

ЗБОРНИК

МАТИЦЕ СРПСКЕ
ЗА ПРИРОДНЕ НАУКЕ

М А Т И Ц А С Р П С К А
P R O C E E D I N G S F O R
N A T U R A L S C I E N C E S

118

NOVI SAD
2010



МАТИЦА СРПСКА
ОДЕЉЕЊЕ ЗА ПРИРОДНЕ НАУКЕ

ЗБОРНИК
МАТИЦЕ СРПСКЕ ЗА ПРИРОДНЕ НАУКЕ

MATICA SRPSKA
DEPARTMENT OF NATURAL SCIENCES
PROCEEDINGS FOR NATURAL SCIENCES

Покренут 1951 / First published in 1951.

Published as *Научни зборник*, серија природних наука until the tenth issue (1955), as the Series for Natural Science from the eleventh issue (1956) — *Зборник за природне науке*, and under its present title since the sixty-sixth issue (1984)

Главни уредници / Editors-in-Chief

Miloš Jovanović (1951), Branislav Bukurov (1952—1969),
Lazar Stojković (1970—1976), Slobodan Glumac (1977—1996), Rudolf Kastori (1996—)

118

Уредништво / Editorial Board

S. GAJIN
V. JANJIĆ
V. JOVIĆ
D. KAPOR
R. KASTORI
L. LEPSANOVIĆ
I. MAKSIMOVIĆ
V. MARIĆ
S. ĆURČIĆ

Consulting Editors

A. ATANASSOV, Bulgaria
P. HOCKING, Australia
A. RODZKIN, Republic of Belarus
M. SIMMONDS, UK
S. STOJILKOVIC, USA
G. SCHILING, Germany
GY. VÁRALLYAY, Hungary

Главни и одговорни уредник / Editor-in-Chief
RUDOLF KASTORI

ISSN 0352-4906 | UDK 5/6(082)

MATICA SRPSKA
PROCEEDINGS FOR
NATURAL SCIENCES

118

NOVI SAD
2010

CONTENTS

САДРЖАЈ

ARTICLES AND TREATISES

ЧЛАНЦИ И РАСПРАВЕ

<i>Čedomir N. Radenović, Ksenija P. Marković, Aleksandar R. Radojčić, Violeta B. Anđelković, Aleksandar J. Kalauzi</i> , Interdependence between oscillations and transients of delayed fluorescence induction processes in the thylakoid membrane of the intact maize leaf — responses to effects of increased temperatures and drought — Међузависност осцилација и транзијената индукционих процеса закасне флуоресценције хлорофила у тилакоидној мембрани интактног листа кукуруза — реакције на деловање виших температура и суше	7
<i>Zoran Ž. Tambur, Igor M. Stojanov, Sonja M. Konstantinović, Dara V. Jovanović, Desanka Cenić-Milošević, Dolores N. Opačić</i> , Multi drug resistance of <i>Campylobacter jejuni</i> and <i>Campylobacter coli</i> to tested antibiotics in strains originating from humans, poultry and swine — Мултирезистенција <i>Campylobacter jejuni</i> и <i>Campylobacter coli</i> на испитивање антибиотике код сојева пореклом из људи, живине и свиња	27
<i>Janja P. Kuzevski, Nada A. Milošević, Saša B. Krstanić, Zora S. Jeličić</i> , Effect of <i>Azotobacter chroococcum</i> on sugar beet and microbial activity of rhizosphere — Утицај <i>Azotobacter chroococcum</i> на усе и микробиолошку активност ризосфере шећерне репе	37
<i>Nataša P. Nikolić, Ljiljana S. Merkulov, Borivoj Đ. Krstić, Slobodanka P. Pajević, Milan K. Borišev, Saša S. Orlović</i> , Variability of acorn anatomical characteristics in <i>Quercus robur</i> L. genotypes — Варијабилност анатомских особина жира код генотипова храста лужњака (<i>Quercus robur</i> L.)	47
<i>Ivana J. Sedej, Marijana B. Sakač, Aleksandra Č. Mišan, Anamarija I. Mandić</i> , Antioxidant activity of wheat and buckwheat flours — Антиоксидативна активност брашна од пшенице и хељде	59
<i>Rudolf R. Kastori, Ivana V. Maksimović, Radovan Z. Marinković, Tijana M. Zeremski-Škorić, Jordana N. Ninčkov, Marina I. Putnik-Delić</i> , Genetic variability of concentration of microelements in wild sunflower species and hybrids — Садржај микроелемената у популацијама дивљих врста сунцокрета и хибрида	69

<i>Borivoj Đ. Krstić, Đuro J. Gvozdenović, Nataša P. Nikolić</i> , Genetic variability of mineral elements concentration in pepper varieties (<i>Capsicum annuum</i> L.) — Генетичка варијабилност концентрације минералних елемената код различитих сорти паприке (<i>Capsicum annuum</i> L.)	79
<i>Rudolf R. Kastori, Ivana V. Maksimović, Tijana M. Zeremski-Škorić, Marina I. Putnik-Delić</i> , Rare earth elements — yttrium and higher plants — Елементи ретких земаља — итријум и више биљке	87
<i>Dejana M. Džigurski, Slobodanka J. Stojanović, Aleksa S. Knežević, Ljiljana M. Nikolić, Branka B. Ljevnaić-Mašić</i> , Vegetation of the classes <i>Hydrochari-Lemnetea</i> Oberd. 1967 and <i>Potametea</i> Tx. et Prsg. 1942 in the Jegrička watercourse (the Province of Vojvodina, Serbia) — Вегетација класа <i>Hydrochari-Lemnetea</i> Oberd. 1967. и <i>Potametea</i> Tx. et Prsg. 1942 у водотоку Јегричке (Војводина, Србија)	99
<i>Ivica G. Đalović, Ivana V. Maksimović, Rudolf R. Kastori</i> , Mechanisms of adaptation of small grains to soil acidity — Механизам адаптације стрних жита на киселост земљишта	107
<i>Vladan D. Mirjanić, Radmila R. Arbutina, Jovan P. Šetrajčić, Ljubiša D. Džambas</i> , Physical properties of thin films on implant-materials — Физичке особине танких филмова на имплант-материјалима	121
<i>Goran S. Marković, Miroslav A. Ćirković, Stevan A. Maletin, Nikolina J. Milošević</i> , A contribution to the data on tench (<i>Tinca tinca</i> L., Cyprinidae, Pisces) distribution in Serbia — Прилог подацима о распрострањењу лињака (<i>Tinca tinca</i> L., Cyprinidae, Pisces) у Србији	127
<i>Marko J. Tucakov</i> , Species composition and number of breeding birds in urban parks in Novi Sad — Састав врста и бројност птица гнезларица у градским парковима у Новом Саду	143
<i>Dragan R. Žikić, Gordana M. Ušćebrka, Dušan S. Gledić, Miodrag I. Lazarević</i> , The influence of long-term sound stress on histological structure of immune organs in broiler chickens — Утицај дуготрајног звучног стреса на хистолошку грађу имунолошких органа код бројлерских пилића	151

Зборник Матице српске за природне науке издаје Матица српска
Излази двапут годишње
Уредништво и администрација: Нови Сад, Улица Матице српске 1
Телефон: 021/420-199
e-mail: zmspn@maticasrpska.org.rs
www.maticasrpska.org.rs
Proceedings for Natural Sciences published by Matica Srpska
Published twice a year
Editorial and publishing office: Novi Sad, Ul. Matice Srpske 1
21000 Novi Sad, Serbia
Phone: +381-21/420-199

The editors of the Matica srpska *Proceedings for Natural Sciences*
Completed the selection for Issue 117/2009 on October 1, 2009
Editorial Staff Secretary: Julkica Boarov
Managing editor: Dr. Slavka Gajin
English text proof-reader: Bojana Filipčev and Vera Vasilčić
Technical design: Vukica Tucakov
Published in March 2010
Publish by: Mladen Mozetić, GRAFIČAR, Novi Sad
Printed by: ALFAgraf, Novi Sad

Публиковање овог броја помогло је
Министарство за науку и технолошки развој Републике Србије
и Покрајински секретаријат за науку и технолошки развој
The edition and printing of the Proceedings has been financially supported by
the Ministry of Science and Environmental Protection of Republic of Serbia
and Provincial Secretariat for Science and Technological Development

CIP — Каталогизација у публикацији
Библиотека Матице српске, Нови Сад

5/6(082)

ЗБОРНИК Матице српске за природне науке = Matica
srpska proceedings for natural sciences / главни и одговорни
уредник Рудолф Кастори. — 1984, св. 66— . — Нови Сад
: Матица српска, Одељење за природне науке, 1984—. —
24 cm

Два пута годишње. — Наставак публикације: Зборник за
природне науке. — Текст на енгл. језику, резимеи на енгл. и
на срп. језику.

ISSN 0352-4906

COBISS.SR-ID 5845250

ISSN 0352-4906



9 770352 490002

*Čedomir N. Radenović^{1, 2}, Ksenija P. Marković¹,
Aleksandar R. Radojčić¹, Violeta B. Anđelković¹
Aleksandar J. Kalauzi³*

¹ Maize Research Institute, Zemun Polje, Belgrade, Serbia

² Faculty of Physical Chemistry, University of Belgrade, Belgrade, Serbia

³ Center for Multidisciplinary Studies, University of Belgrade, Belgrade, Serbia

INTERDEPENDENCE BETWEEN OSCILLATIONS AND TRANSIENTS OF DELAYED FLUORESCENCE INDUCTION PROCESSES IN THE THYLAKOID MEMBRANE OF THE INTACT MAIZE LEAF — RESPONSES TO EFFECTS OF INCREASED TEMPERATURES AND DROUGHT

ABSTRACT: Standard induction processes of delayed fluorescence (DF) of chlorophyll (induction signals) occur when an intact leaf segment of maize inbreds and hybrids is kept in the phosphoroscope darkroom for more than 15 minutes ($\tau > 15$ min), and at the same time the leaf is illuminated with the intermittent white light.

Resolved induction processes of DF chlorophyll into transients: A, B, C, D and E occur when the intact leaf segment of maize inbreds and hybrids is kept in the phosphoroscope darkroom for a significantly shorter period ($500 \text{ s} > \tau > 30 \text{ s}$), with the time rate t of 30 s, prior to its illumination with the intermittent white light. Induction transients: A, B, C, D and E are characterised with the time of their generation: $t_A = 31 \pm 6 \text{ ms}$ (A), $t_B = 5 \pm 0,5 \text{ s}$ (B), $t_C = 15 \pm 5 \text{ s}$ (C), $t_D = 360 \pm 20 \text{ s}$ (D) and $t_E = 670 \pm 35 \text{ s}$ (E), dynamics of changes in transients intensities (I_A , I_B , I_C , I_D and I_E) and mechanisms of their generation. The induction processes of chlorophyll DF of the intact leaf of maize inbreds and hybrids resolved into transients: A, B, C, D and E are accompanied by the occurrence and different levels of activation energy (E_a , kJ mol^{-1}) that correspond to critical temperatures ranging from 28 to 33°C.

The generation mechanisms of induction transients A, B, C, D and E classify them into two groups. Transients A and B are of a physical character, while the transients: C, D, and E are of a chemical character.

It is shown that the generation of the induction transients: B, C, D and E simultaneously follows establishing of the oscillations of induction processes of the DF chlorophyll. Oscillating of induction processes of DF chlorophyll is explained by the ion (K^+ , Na^+ , H^+ , Cl^-) transport mechanism across the thylakoid membrane of the intact leaf of maize inbreds and hybrids grown under conditions of air drought, increased temperatures and water deficiency in the medium.

KEY WORDS: *Zea mays*, intact leaf, thylakoid membrane, induction processes, transients, oscillations, critical temperature, activation energy, delayed chlorophyll fluorescence

INTRODUCTION

In recent times, binding complex processes of fundamental sciences with multidisciplinary ones has become the necessity. Such attempts have been made between breeding, photosynthesis, biophysical chemistry and fluorescence in maize inbreds and hybrids. The present study analyses the development of dominant processes of the stated scientific disciplines and areas of their mutual dependence and application.

Maize breeding has been intensively developed during the last 60 years. Because of such an activity, over 1,100 maize hybrids for grain and silage have been developed. Contemporary technical and technological prerequisites for conducting a modern process of breeding were provided (Duvick, 1984, Trifunović, 1986, Ivanović et al., 1995, Radenović and Somborac, 2000). Regardless of such a colossal success in maize breeding, eagerness and enthusiasm of the total research have not been slowing down. The search for new methods and exact approaches in the completion and enrichment of the research within maize breeding and seed production was continued.

The development of maize photosynthesis was quite different. Namely, although photosynthetic processes are very spread, highly productive in their intensity, very complex in their nature, and vastly studied in their scientific actuality, their application in maize breeding is still insignificant. It is almost impossible to make a distinct, direct interrelationship between photosynthesis and breeding. Such a state is probably a consequence of the existence of several functional interrelations that unify conformational and dynamic changes within chloroplasts and their thylakoid membranes, on the one hand, and effects of numerous environmental stress factors on them, on the other hand.

Biophysical chemistry contributed, to a great extent, to connecting photosynthetic processes in the thylakoid membrane to the processes of fluorescence spectroscopy and chemical kinetics (Rubin et al., 1988, Radenović et al., 1981, 1985).

The delayed chlorophyll fluorescence (DF) phenomenon can be described as an occurrence of luminescence (bioluminescence) within the red range of the visible spectrum produced by plant systems: bacteria, algae and higher plants (maize) immediately upon their intermittent illumination (excitation) (in case of maize upon illumination of the intact leaf) (Radenović et al., 1981, 1985; Radenović, 1992, 1994, 1997, Marković et al., 1996, 1999). DF was discovered by Strehler and Arnold (1951) in their attempt to reveal a nature of induction illumination in a form of bioluminescence. Numerous studies, especially those of the last 20 years (Jursnić and Govinidje, 1982, Jursnić, 1986, Marković et al., 1996, 1999, Veselovski and Veselova, 1990, Radenović, 1992, 1994, 1997, Radenović et al., 1994a, 1994b, Kaluzi et al. 2006), revealed the direct connection between DF and photosynthetic processes, in which DF was considered as an unavoidable indicator — a susceptible „probe” for experimental photosynthetic studies in the intact leaf of maize inbreds and hybrids (Rade-

nović, 1992, 1994, 1997, Radenović et al., 1994a, 1994b, Radenović and Jeremić 1996, Marković et al., 1987, 1993, 1996, 1999). Today, as well as, in the near future, DF shall be an efficient tool, i.e. a methodological approach in studies of certain, mostly very complex photoprocesses in the light phase of photosynthesis. In relation to this approach, induction processes of chlorophyll DF, their resolution into several mutually dependent transients, generating of oscillations and rhythms and their correlation are considered as actual scientific issues. Within the scope of the stated, question arise about the activation energy and critical temperatures within activities of the total induction processes of chlorophyll DF. Hence, these issues and their interrelations are an objective of the present study.

MATERIAL AND METHODS

The studies were done with the intact leaf of the selected hybrids: ZPDC 360, ZPSC 46A, ZPSC 704 and ZPSC 71, and inbred lines: ZPR70ž and Oh43, tolerant and resistant to effects of increased temperatures and drought. The test plants were grown in pots in the glass-house under conditions of controlled and regulated air drought, relative humidity, temperatures and a reduced amount of water in the medium. Changes in the temperature were controlled and it reached the level of 38°C, although mostly ranged from 28 to 38°C. Plants were grown under such conditions through all stages of their growth and development. Recordings were done for four years in June, July and August. Maize plants were brought from the glass-house into the laboratory during morning hours (between 7 a.m. and 8 a.m.). Plants sampled in the glass-house were transversally cut in the ground internode. In the laboratory, plants were internode lengthwise placed in water. Two hours prior to the bioluminescence experiment, the plants were kept under the black ball glass. A segment of ear intact leaves was taken from such plants and placed into a chamber of the Becquerel phosphoroscope. These studies were done with 40 plants each of maize hybrids and maize inbreds.

The non-invasive photosynthetic fluorescence method was used to measure the intensity of the chlorophyll DF induction process. These measures also included the resolution of the induction curve into the transients: A, B, C, D and E, as well as, recording of the generation of oscillations and rhythms.

The improved non-invasive photosynthetic fluorescence method used to measure induction processes of chlorophyll DF is schematically presented in Fig. 1. This block scheme of the photosynthetic fluorescence method was developed at the Maize Research Institute, Zemun Polje. Measurements of changes in the induction processes of chlorophyll DF were performed after a method that had been both, in principle and details, described in previous papers (Radenović et al., 1981, 1985, Radenović, 1985, 1992, 1994, 1997, Marković et al., 1996).

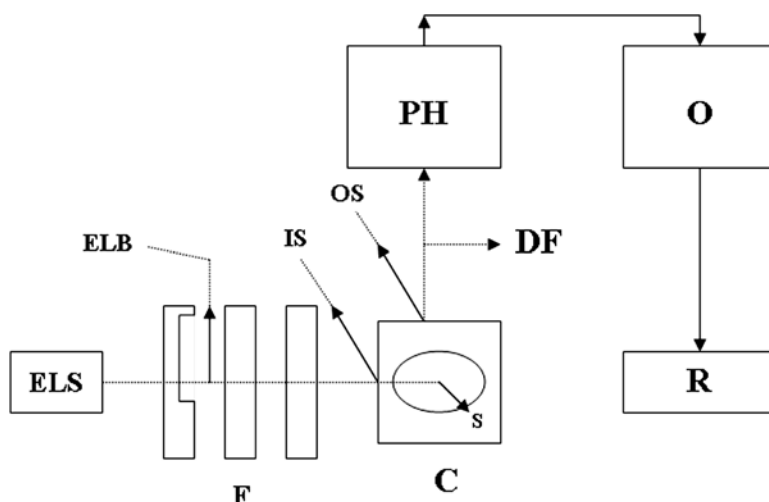


Fig. 1. — Experimental setup of the photosynthetic fluorescence method and measuring equipment for delayed chlorophyll fluorescence: **C** — dark chamber with a sample stand; **s** — sample (intact leaf segment), **F** — filters, **ELS** — excitation light source, **PH** — photo-multiplier; **O** — oscilloscope, **R** — printer, **ELB** — excitation light beam, **DF** — luminescent light (delayed fluorescence), **IS** — input dark chamber slot, **OS** — output dark chamber slot

RESULTS

1. *Standard induction processes*

Standard induction processes of delayed fluorescence (DF) of chlorophyll (induction signals) occur when an intact leaf segment of maize inbreds and hybrids is kept in the phosphoroscope darkroom for more than 15 minutes ($\tau > 15$ min), and at the same time is illuminated with the intermittent white light. Results of induction processes of chlorophyll DF are presented in Fig. 2. Under given conditions the induction curve of chlorophyll DF does not provide the possibilities of oscillations generation or the formation of transients on it: A, B, C, D and E of induction processes. The registered form of the standard induction curve of chlorophyll DF has four typical parts. The part „a” is formed very quickly, for about 1—2 s, the part „b” designates the maximum level of the induction curve intensity, the part „c” is gradually formed in the exponential trend, and at the end, the part „d” represents the stationary level of the induction curve of chlorophyll DF, Fig. 2.

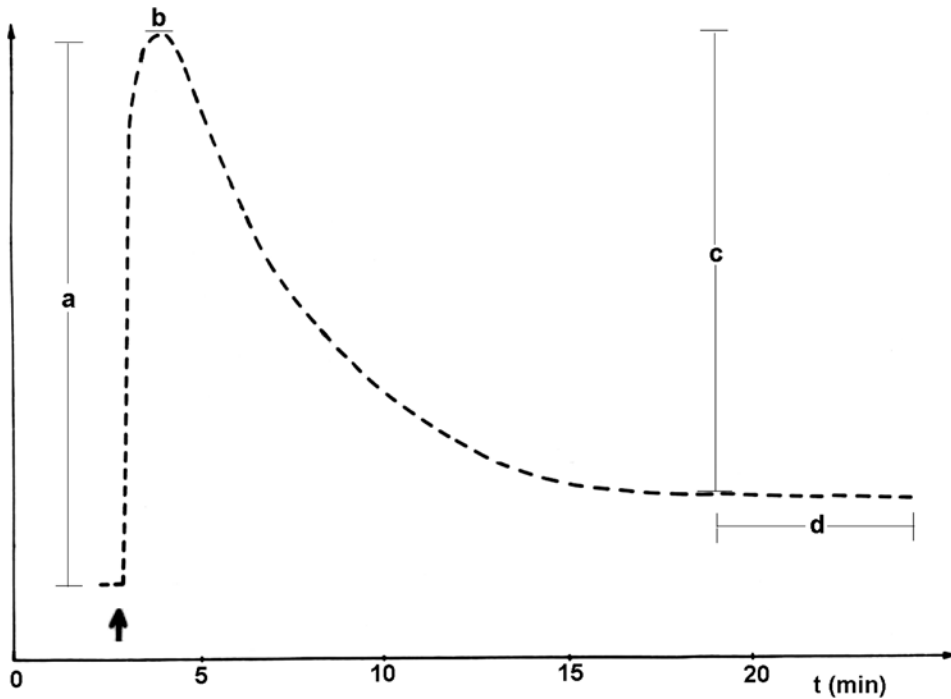


Fig. 2. — Schematic illustration of standard induction curve of chlorophyll DF typical for the intact leaf of maize inbreds and hybrids kept in the phosphoroscope darkroom longer than 15 minutes ($\tau > 15$ min)

2. Resolution of delayed chlorophyll fluorescence induction processes into transients

The resolution of the induction processes of chlorophyll DF into transients: A, B, C, D and E and the appearance of oscillations occur when the intact leaf segment of maize inbreds and hybrids is kept in the phosphoroscope darkroom for a significantly shorter period ($500 \text{ s} > \tau > 30 \text{ s}$) prior to its illumination with the intermittent white light, Fig. 3.

Results of the experimental resolution of induction processes of chlorophyll DF into transients: A, B, C, D and E are presented in Fig. 4—6 and Tab. 1.

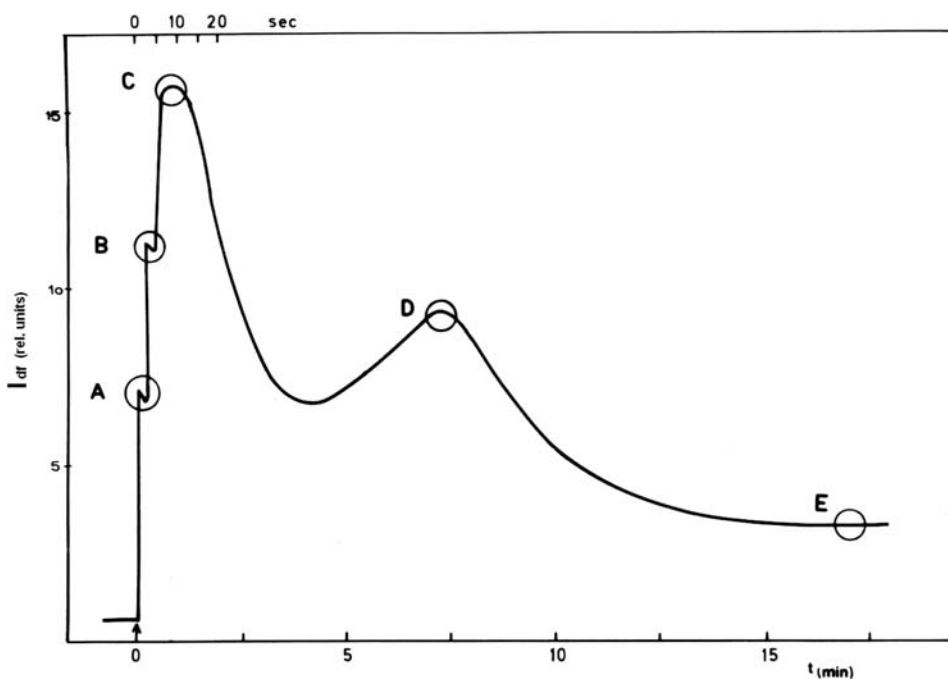


Fig. 3. — Schematic illustration of possible sites of the oscillation generation and the formation of transients: A, B, C, D, E of the induction processes of delayed chlorophyll fluorescence in the intact leaf of maize inbreds and hybrids

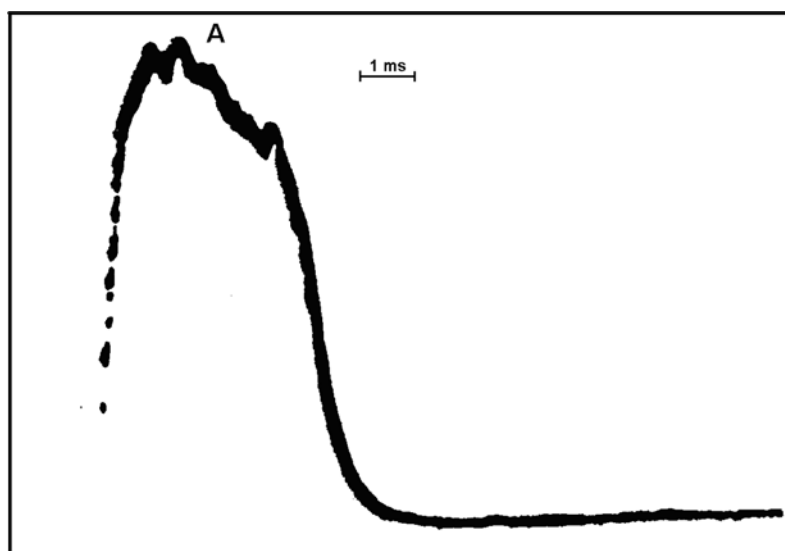


Fig. 4. — Experimental detection of the transient A of the induction processes of delayed chlorophyll fluorescence in the intact leaf of maize inbreds and hybrids with the use of the storage oscilloscope and a time scale of 1 ms

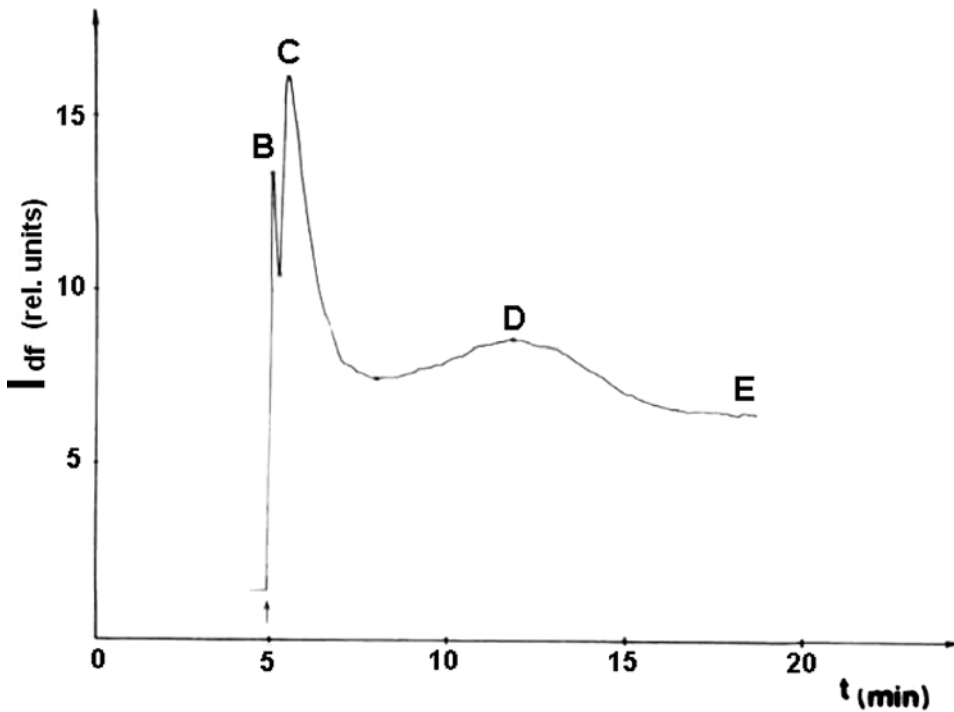


Fig. 5. — Typical presentation of the experimental results on the resolution into transients: B, C, D and E of the induction processes of delayed chlorophyll fluorescence in the intact leaf of maize inbreds and hybrids for the time t in seconds ($500 \text{ s} > \tau > 30 \text{ s}$)

Tab. 1. — Time parameters of transients, A, B, C, D and E of the induction processes of delayed chlorophyll fluorescence in the intact leaf of studied maize inbreds and hybrids at 25°C

Time of generation of transients: A, B, C, D and E	Transients of the induction processes of delayed chlorophyll fluorescence in the intact leaf of maize inbreds and hybrids				
	A: t_A , ms	B: t_B , s	C: t_C , s	D: t_D , s	E: t_E , s
Time of the beginning of transient generation	2—4	0.8—1.0	9—11	110—130	180—240
Time of transient generation with the maximum intensity	28—32	6—7	13—17	180—200	380—480 (decrease)
Longest time of transient generation	75—80	8—9	21—23	480—500	8400
Average time of transient generation	31 ± 6	5 ± 0.5	15 ± 5	360 ± 20	670 ± 35

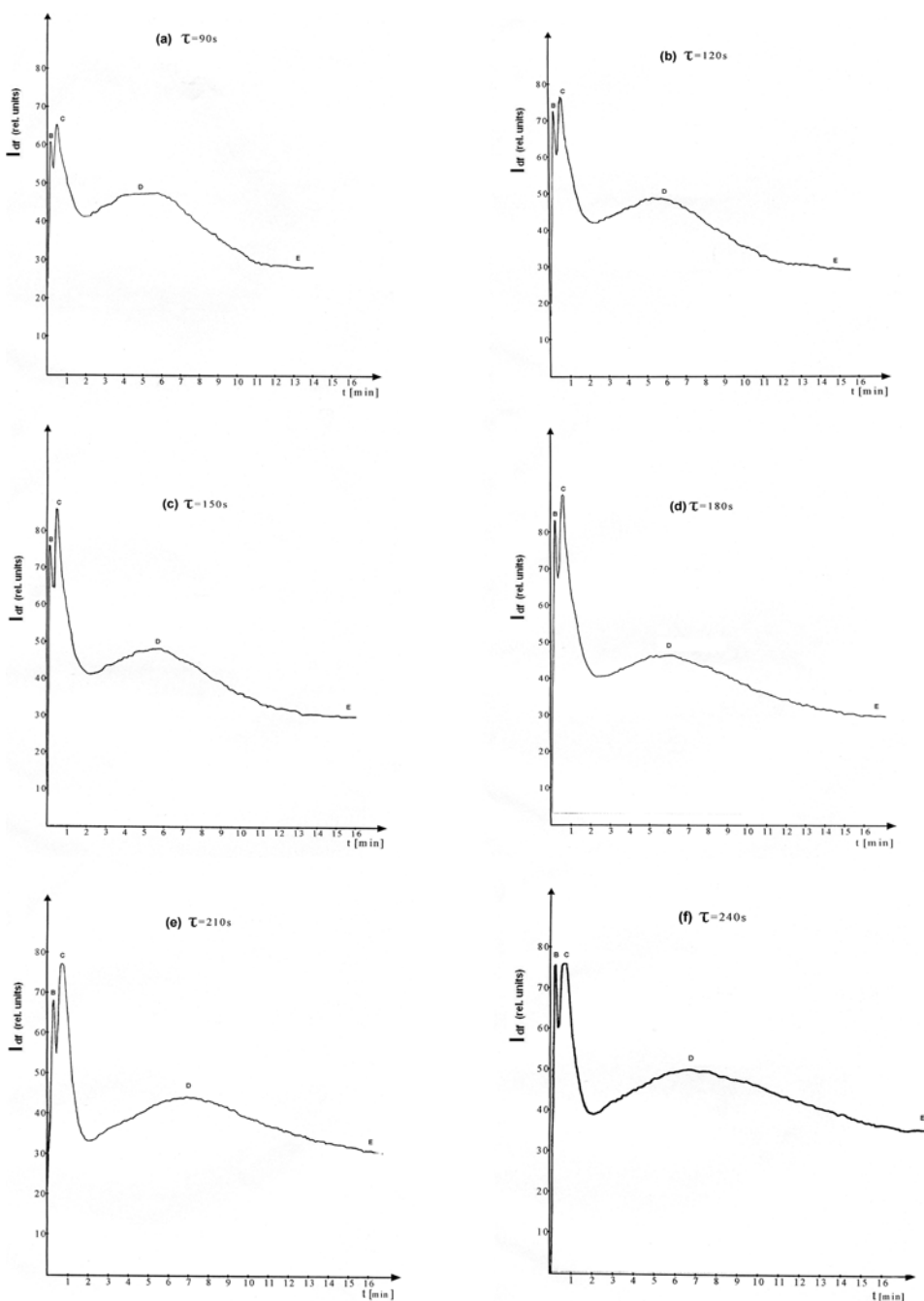


Fig. 6a-f — Experimental results on the resolution of the induction curve of delayed chlorophyll fluorescence into transients: B, C, D and E for the following values of t : 90s, 120s, 150s, 180s, 210s and 240s

3. Dynamics of changes in intensities of transients of delayed chlorophyll fluorescence induction processes in dependence on temperature

Obtained results on changes in the intensity of induction transients: B, C, D and E in observed maize inbreds and hybrids in dependence on temperature are presented in Fig. 7 and Tab. 2.

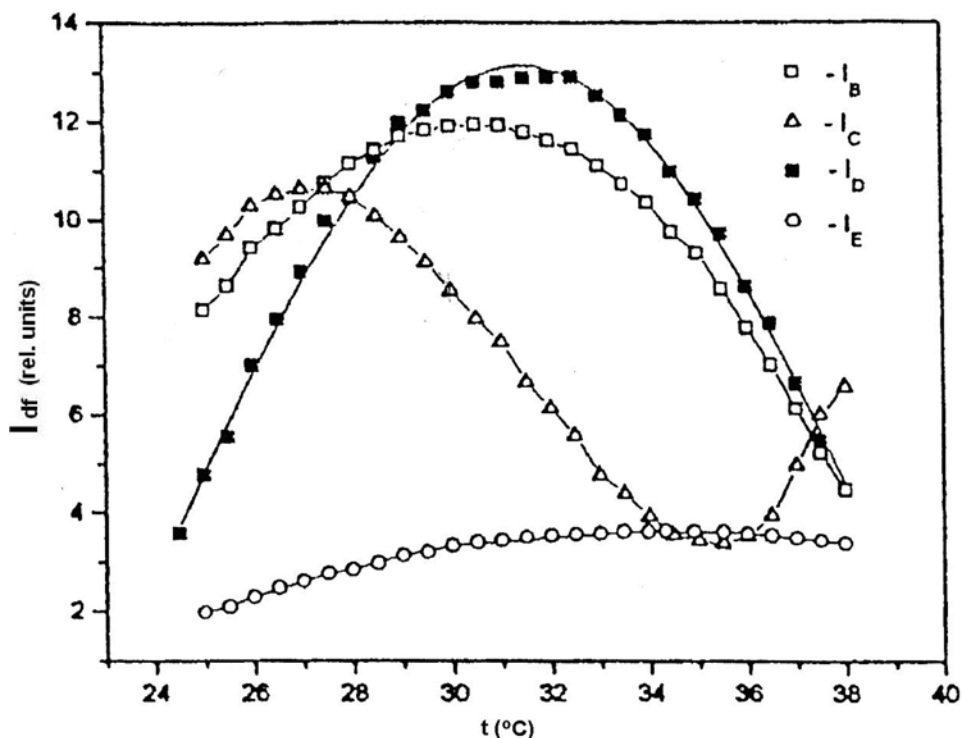


Fig. 7. — Dynamics of changes in intensities of transients B, C, D and E of delayed chlorophyll fluorescence induction processes in dependence on temperature

Tab. 2. — Temperatures of the transition period of transients: B, C, D and E of delayed chlorophyll fluorescence induction processes in the intact leaf of studied maize inbreds and hybrids

Previous intact leaf dark period (τ , s)	Temperatures of transition period, t °C			
	Transient B	Transient C	Transient D	Transient E
30	33.3 $\pm$ 0.2	28.5 $\pm$ 2.5	31.6 $\pm$ 0.2	30.8 $\pm$ 0.2
60	23.4 $\pm$ 0.3	32.7 $\pm$ 3.0	25.4 $\pm$ 0.3	22.0 $\pm$ 0.3
90	19.5 $\pm$ 0.2	25.8 $\pm$ 0.3	26.5 $\pm$ 0.2	20.5 $\pm$ 0.4
120	17.9 $\pm$ 0.2	25.0 $\pm$ 0.5	22.5 $\pm$ 0.2	21.0 $\pm$ 0.4
150	24.7 $\pm$ 0.4	26.3 $\pm$ 0.4	22.5 $\pm$ 0.2	25.0 $\pm$ 0.3
180	24.0 $\pm$ 0.3	26.3 $\pm$ 0.3	23.1 $\pm$ 0.2	23.8 $\pm$ 0.5
210	24.3 $\pm$ 0.3	31.0 $\pm$ 0.4	22.0 $\pm$ 0.2	22.2 $\pm$ 0.3
240	26.1 $\pm$ 0.3	32.0 $\pm$ 0.4	23.8 $\pm$ 0.2	22.8 $\pm$ 0.5

Fig. 7 shows that changes in the intensities of transients B and D are similar. In the beginning, the intensity of the transient B (I_B) increases at the temperature range of 24—29°C, then reaches and maintains the maximum value within the temperature range of 29—32°C, and after that, it gradually decreases at the temperature range of 32—38°C. The intensity of the transient D (I_D) shows the similar trend, but its parabola has a somewhat steeper slope (Fig. 7). On the other hand, the dynamics of changes in the intensity of the transient C (I_C) is different. It is an inverse sigmoid (in the beginning, the intensity of the transient C increases at the temperature up to 26°C, when it reaches its maximum, then it gradually decreases at the temperature of 35.5°C at which it reaches its minimum and then again it increases up the temperature range of 35.5—38.0°C) (Fig. 7). Changes in the intensity of the transient E (I_E) are standard and expected.

4. Activation energy of transients of delayed chlorophyll fluorescence induction processes

Tab. 3 presents results on the activation energy (E_a) of transients B, C, D and E of chlorophyll DF induction processes

Tab. 3. — Activation energy of transients: B, C, D and E of delayed chlorophyll fluorescence induction processes in the intact leaf of studied maize inbreds and hybrids

Previous intact leaf dark period (τ , s)	Activation energy (E_a , kJ mol ⁻¹)			
	Transient B	Transient C	Transient D	Transient E
30	65.37	83.69	266.98	32.08
60	32.90	23.80	39.66	26.28
90	51.81	14.03	27.83	53.68
120	52.96	47.68	63.68	92.53
150	30.07	68.52	93.53	106.62
180	25.97	75.05	116.27	106.32
210	22.35	34.43	132.19	92.74
240	21.60	30.09	131.80	74.49

The dynamics of changes in the intensities of transients: I_B , I_C , I_D and I_E (Fig. 7), changes in the phase transition temperature (Tab. 2) and the activation energy (Tab. 3) do not proceed according to a certain established rule, which indicates the rapidity of their occurrence and the total complexity of photosynthetic and fluorescence system.

5. The correlations of transients with oscillations in the delayed chlorophyll fluorescence induction processes

Experimental results on dependency of intensities of transients B, C, D and E and oscillations in chlorophyll DF induction processes in studied maize inbreds and hybrids are presented in Fig. 8—11.

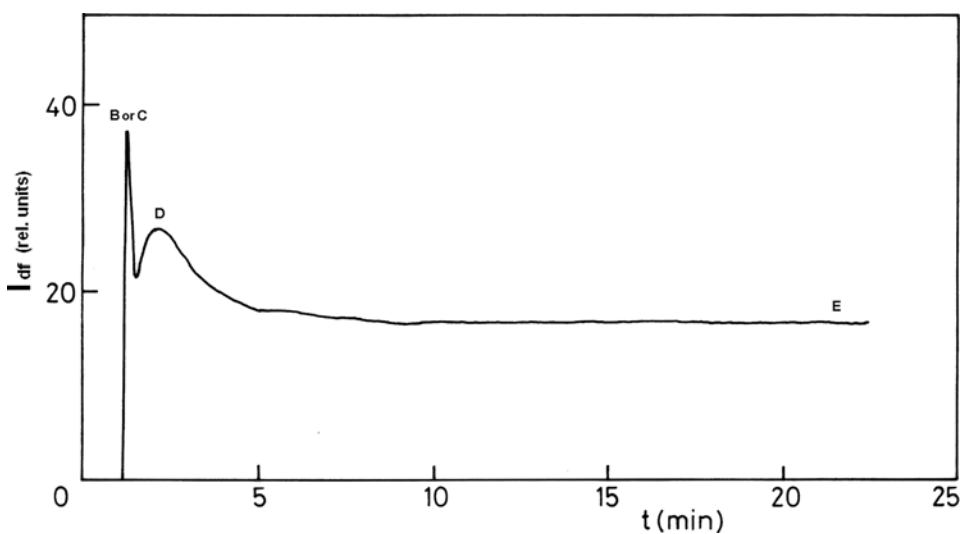


Fig. 8. — Generation of the standard induction curve of delayed chlorophyll fluorescence with transients B or C, D and E in the intact leaf of studied maize inbreds and hybrids grown under common physiological conditions

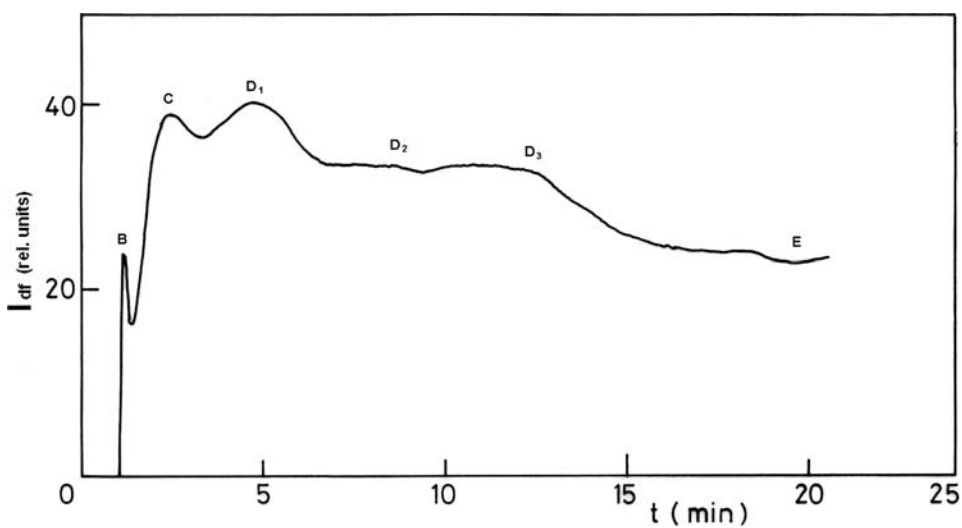


Fig. 9. — Generation of the excited state of the induction curve of delayed chlorophyll fluorescence with transients B, C, D₁, D₂, D₃ and E in the intact leaf of studied maize inbreds and hybrids grown under conditions of weak air drought

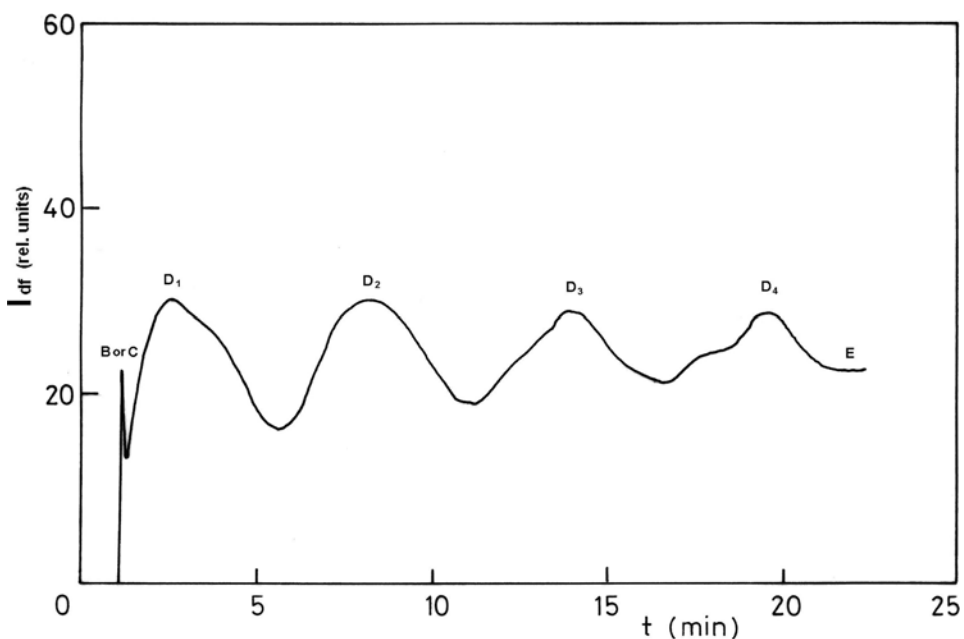


Fig. 10. — Generation of the fluctuation of induction curve of delayed chlorophyll fluorescence with transients B or C, D₁, D₂, D₃, D₄ and E in the intact leaf of studied maize inbreds and hybrids grown under conditions of severe air drought

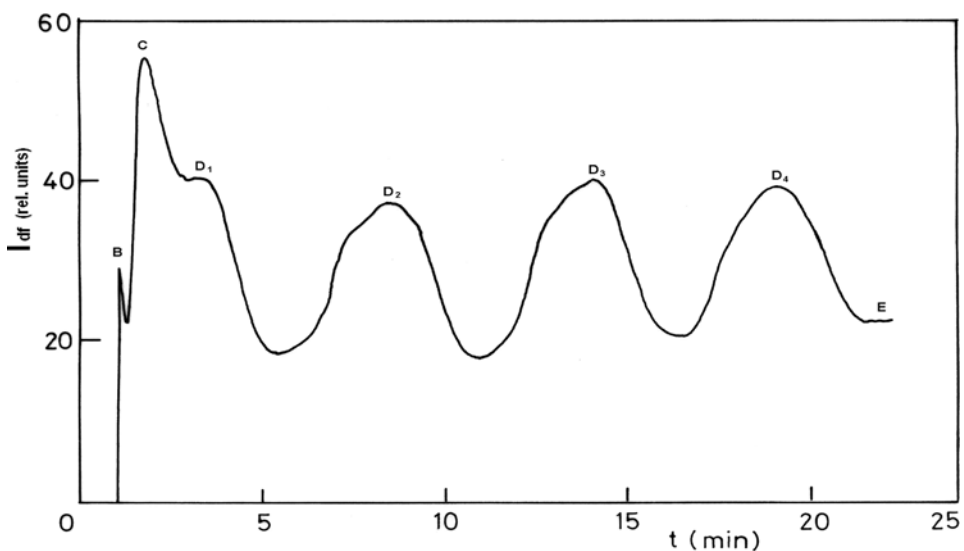


Fig. 11. — Generation of the oscillations of the induction curve of delayed chlorophyll fluorescence with transients B, C, D₁, D₂, D₃, D₄ and E in the intact leaf of studied maize inbreds and hybrids grown under conditions severe air drought and reduced water in the medium

If observed maize inbreds and hybrids are grown under conditions of different intensities of air drought, decreased relative humidity and higher temperatures, obtained fluorescence responses of induction processes will be not only with transients but also with oscillations.

According to such studies the intensities of transients: B, C, D and E of chlorophyll DF induction processes are connected to the induction and the establishment of processes of instability, excited state, fluctuations and oscillations in thylakoid membranes in the intact leaf of maize inbreds and hybrids. Such a response was obtained in observed maize inbreds and hybrids that were tolerant and resistant to drought, Fig. 8—11.

DISCUSSION

In the experimental resolution of chlorophyll DF induction processes, transients B, C, D and E were initially revealed by the application of the standard procedures for chlorophyll DF detection (Radeno vić et al., 1981). Much later, the transient A was revealed (Radeno vić, 1997). It was much more difficult to be detected. It was necessary to provide a storage oscilloscope and to connect it to the equipment set up to register chlorophyll DF induction processes. Induction transients: A, B, C, D and E are characterised by their general and physical and chemical parameters, i.e. time of their generation (t_A , t_B , t_C , t_D and t_E), dynamics of changes in transient intensities (I_A , I_B , I_C , I_D and I_E) and mechanisms of their generation, Tab. 1 and Fig. 7.

Generation mechanisms of transients A, B, C, D and E

The generation mechanism of the transient A is explained by its connection to the primary photochemical act that predominantly affects the formation of the induction dipole within the photosystem II reaction centre. The generation of this transient is a result of the orientation of electric dipoles in the thylakoid membrane under effects of the illumination with the intermittent white light that excites them. The dipole orientation induces an electric field. Such dipoles define the transient A pointing out to its physical nature and its effects on the delayed chlorophyll fluorescence induction processes.

The transient B generation is primarily attributed to the formation of the electrochemical gradient across the thylakoid membrane after the illumination of the intact leaf segment with the intermittent white light. More precisely, this transient strongly depends on the electrical component of the electrochemical gradient (DF). The transient B is a direct product of the thylakoid membrane excitation under the illumination with the intermittent white light. It is believed that a photodipole interacts with other membranous structures and that this interaction causes the excitation that is in fact expressed as the transient B of the chlorophyll DF induction processes. As this interaction is rapid, more rapid than chemical reactions, its basis is not purely chemical but much more physical.

It is considered that the transient C generation depends on photosensitive reactions centres. These centres depend on t (previous intact leaf dark period), i.e. their number increases in dependence on t , and then conformational and functional changes occur in certain protein structures that determine mutual position of acceptors (Chl) in the reaction centre. This transient has kinetic characteristics similar to the transient B as it is susceptible to the presence of valomycin, which is capable to eliminate the electrochemical gradient (Radeno-
 nović, 1997, Radenović et al., 2003, 2007, Marković et al., 1999; Kalauzi et al., 2006). The transient C depends on the electron transport between two photosystems (PS II and PS I). In particular, the intensity of this transient increases with the increase of the accumulation of X^- (secondary PS I electron acceptor in the reduction form until it reaches its peak that corresponds to the electrochemical gradient maximum). The X^- accumulation corresponds to the accumulation of PS II P_680^- and Q^- . Similarly, the drop in the transient C intensity is caused by the decrease of the electrical component of the electrochemical gradient ($\Delta\Psi$), and cation diffusion from thylakoids. It is in fact, the indication of the dissipation of the electrochemical gradient. Kinetic characteristics of these transients depend on the duration of the dark period. The prolongation of the dark period leads to the decrease in the transient C intensity and simultaneously to the increase in the transient D intensity.

The transient D of the chlorophyll DF induction curve appears, and therefore it is easily detected, only when its observation is performed outside of the optimal temperature range ($28^\circ\text{C} < \tau < 16^\circ\text{C}$). Hence, this transient primarily depends on the temperature. The transient D also depends on the electrochemical gradient, particularly on its proton component (DpH). The formation of the electrochemical gradient relates to „energisation” of thylakoids, which are dynamic entities whose structures and functional properties response to environmental stimuli. Therefore, if the transient D amplitude increases, corresponding changes in the thylakoids structure will occur in the course of protonation and phosphorylation. In case of the transient D drop, changes in thylakoids structure cause mutual nearing of photosystems I and II and alleviate the intersystem electron transmission. It was determined that transients C and D can be kinetically expressed by two consecutive chemical reaction of the first order with different rate constants, Tab. 4 (Radeno-
 nović et al., 1985).

Tab. 4. — Rate constants of chemical reactions in the thylakoid membrane of the intact leaf of maize inbreds and hybrids for transients C and D of delayed chlorophyll fluorescence induction processes at 25°C

Intact leaf of maize inbreds and hybrids	Rate constants of chemical reactions	
	Transient C	Transient D
ZPDC 370	$k_{C1} = 5,13 \times 10^{-3}s^{-1}$ $k_{C2} = 2,41 \times 10^{-3}s^{-1}$	$k_{D1} = 3,98 \times 10^{-3}s^{-1}$ $k_{D2} = 2,04 \times 10^{-3}s^{-1}$
ZPSC 46A	$k_{C1} = 5,41 \times 10^{-3}s^{-1}$ $k_{C2} = 6,13 \times 10^{-3}s^{-1}$	$k_{D1} = 4,88 \times 10^{-3}s^{-1}$ $k_{D2} = 2,10 \times 10^{-3}s^{-1}$
ZPSC 704	$k_{C1} = 2,60 \times 10^{-3}s^{-1}$ $k_{C2} = 8,30 \times 10^{-3}s^{-1}$	$k_{D1} = 3,70 \times 10^{-3}s^{-1}$ $k_{D2} = 3,20 \times 10^{-3}s^{-1}$
ZPSC 71	$k_{C1} = 2,82 \times 10^{-3}s^{-1}$ $k_{C2} = 3,16 \times 10^{-3}s^{-1}$	$k_{D1} = 3,55 \times 10^{-3}s^{-1}$ $k_{D2} = 4,28 \times 10^{-3}s^{-1}$
ZPR70ž	$k_{C1} = 4,80 \times 10^{-3}s^{-1}$ $k_{C2} = 6,10 \times 10^{-3}s^{-1}$	$k_{D1} = 3,50 \times 10^{-3}s^{-1}$ $k_{D2} = 3,20 \times 10^{-3}s^{-1}$
Oh 43	$k_{C1} = 5,10 \times 10^{-3}s^{-1}$ $k_{C2} = 6,30 \times 10^{-3}s^{-1}$	$k_{D1} = 4,60 \times 10^{-3}s^{-1}$ $k_{D2} = 2,90 \times 10^{-3}s^{-1}$

The stationary level is regularly established in the course of recording of all induction signals of delayed chlorophyll fluorescence. In case of its dissipation, this level is designated as the transient E. The stationary level of the chlorophyll DF induction curve is established by the drop of the electrochemical gradient across the thylakoid membrane and it maintains the slow changes in the intensity of the chlorophyll DF induction processes. Furthermore, the stationary level of the chlorophyll DF induction curve generally rises with the temperature increase. However, this level depends on the state of photosynthetic structures in observed intact leaves of maize inbreds and hybrids. Under particular conditions including effects of environmental factors, the transient E can take oscillatory properties (Radenović et al., 1981).

The correlations of transients with oscillations in induction processes

Studies on oscillatory phenomena in biological systems, and especially in photosynthetic structures, are relatively a novel subject matter. A great number of issues within the field of oscillatory phenomena have been just set up, initiated or only processed to some extent. Namely, a matter of dispute concerns the correlation of transients of chlorophyll DF induction processes with provoking and establishing instabilities, excited states, fluctuations and oscillations in thylakoid membranes of segments of the maize intact leaf. Such a response was obtained in maize inbreds and hybrids that were tolerant and resistant to drought. The test plants were grown in pots with soil in the glass-house under conditions of controlled and regulated air drought, lower relative humidity, temperature and a reduced amount of water in the medium.

Studying chlorophyll DF induction processes in the intact leaf of maize grown under common and physiological conditions the obtained response was in a form of a chlorophyll DF induction curve in which transients were only

partially expressed, Fig. 8. Instabilities, excited states, fluctuations and oscillations were not detected in them. However, if observed maize inbreds and hybrids had been grown under conditions of weak air drought then fluorescence responses with rhythmical characteristics were obtained and significantly differed from the common chlorophyll DF induction curve. Such a response with the oscillatory characteristics of chlorophyll DF induction processes can have an excited state of the induction curve that remains within the region of its stationary level, which can be affected by transients, Fig. 9. Rhythms can occur in the form of fluctuations of the chlorophyll DF induction curve and can be significantly affected by transients, Fig. 10. At the end, oscillations of the chlorophyll DF induction curve can be expressed in a form of a regular oscillation of the delayed chlorophyll fluorescence stationary level, Fig. 11.

The correlation of transients of chlorophyll DF induction processes with phenomena of instabilities, fluctuations and oscillations is difficult to explain. However, it is believed that transients B, C and D initiate and provoke the stated phenomena (Radenović et al., 1981). Two explanations of their correlations will be briefly presented in this paper. The first explanation is based on the existence of structural and functional regulation within the system of chloroplast of the maize intact leaf in the process of its formation. In other words, this explanation is based on the existence of active reversible links in the system functioning in the rhythmic fluctuating regime. It means that when a photosynthetic apparatus of the intact leaf functions under conditions that it depends on the environmental factors, a periodicity (rhythmicity) of the temporal dependence of structural and functional parameters of the maize leaf occurs.

The starting point of the second explanation is that the phenomena of instabilities, fluctuations and oscillations in the assumed liquid mosaic membranous model can be correlated with the excited state of the thylakoid membrane. The excited state of the thylakoid membrane implies complex conformation changes, and by it, significant functional changes. Namely, proteins migrate and oscillate on the surface of the thylakoid membrane (changes in the surface charge density) and provoke transport process of K^+ , Na^+ , H^+ and Cl^- (Radenović, 2001). It is quite possible to expect that changes in ions within and across the thylakoid membrane reflect on the stability of the stationary level of the chlorophyll DF induction curve in such a way that it acquires properties of the oscillator. It should also be mentioned that one of the pathways of normalising the structure and function of the excited thylakoid membrane leads via the process of phospholipid oxidation, and then via a process of resynthesis of non-oxidative products. These facts are also favoured by the information that the active transport and passive exit of cations and anions are closely related to the periodicity of the hydrolysis and resynthesis of phospholipids, as well as, that they affect activities of macroenergetic compounds (ATP and ADP) in the thylakoid membrane, which can express instabilities, fluctuations and can assume the property of the oscillator and therefore it can transmit them on the chlorophyll DF induction processes (Radenović et al., 1981; Radenović, 2001).

CONCLUSION

According to gained and presented results it can be concluded that the improved photosynthetic-fluorescence method can be successfully applied in the studies on chlorophyll DF induction processes that are resolved into transients A, B, C, D and E and in which oscillations can be generated.

The interdependence of oscillations and induction transients A, B, C, D and E occurs in the intact leaves of studied maize inbreds and hybrids grown in the glass-house under different conditions of controlled and regulated air drought, different % of relative humidity, temperature effects and the water deficiency in the medium.

The following properties of the chlorophyll DF induction processes were determined: this study presents conditions under which standard induction processes of chlorophyll DF were established in the intact leaf of maize inbreds and hybrids and conditions under which the induction signal resolved into transients. The existence of transients A, B, C, D and E was identified. Temporal, physical and chemical parameters for stated transients were shown in the present study. Mechanisms of their occurrence were explained.

The following properties of oscillations of the chlorophyll DF induction processes were determined: this study presents conditions under which the excited state occurred, fluctuations appeared and oscillations were established in the thylakoid membrane of the intact leaf of maize inbreds and hybrids; the hypothesis on the mechanism of the generation of oscillations; the interdependence of the generation of oscillations and transients of chlorophyll DF induction processes was explained in the present study.

ACKNOWLEDGMENT

These 30-year studies encompassing the development of methods, experimental investigations and theoretical discussions were mainly supported by the Maize Research Institute, Zemun Polje, and partially by the Ministry of Science of Serbia and Yugoslavia (projects: 03E22, 12E11, 143043, 142025).

REFERENCES

- Du Vick, D. N. (1984): *Genetic contribution to yield gains of U.S. hybrid maize, 1930—1980*. In: W. R. Fehr, ed. „Genetic contributions to yield gains of five major Crop Plants”. CSSA, Spec. Publ. 7, pp. 15—47, CSSA and ASA, Madison, WI.
- Ivanović, M., R. Petrović, G. Drinić, V. Trifunović, L. Kojić, M. Vuković, M. Mišović, G. Radović, D. Ristanović, Z. Pajić, B. V. Trifunović, D. Jelovac (1995): *Pedeset godina selekcije ZP hibrida kukuruza*. In: „Oplemenjivanje, proizvodnja i iskorišćavanje kukuruza — 50 godina Instituta za kukuruz „Zemun Polje”, Beograd.

- Jursnić and Govindjee (1982): *Effects of hydroxylamine and silicomolybdate on the decay in delayed light emission in the 6–100 μ s range after a single 10 ns flash in pea thylacoides*, Photosynthesis Research 3 (3): 161–177.
- Jursnić, P. (1986): *Delayed fluorescence: Current Concepts and Status*. In: „Light Emission by plants and Bacteria”, Ed: Govindjee, Ames and Fork CD, Academic Press, Orlando, FL. USA, pp. 291–328.
- Kalauzi, A., D. Marković and Č. Radenović (2006): *Transients of Delayed Fluorescence Induction Signal and Photosynthetic Antennas: A Possible Relationship. Mathematical Modeling Approach*. Russian Journal of Plant Physiology Vol. 53, No. 3, pp. 289–297.
- Marković, D. Z., Kalauzi, A. and Radenović, Č. N. (2001): *Delayed fluorescence induction transients: mathematical modelling based on the chosen kinetic models*, Gen. Physiol. Biophys., Vol. 20, pp. 303–313.
- Marković D., Č. Radenović, L. Rafailović, S. Žeraić, M. Marković (1999): *Temperature dependence of delayed fluorescence induction curve transients*. General Physiol. Biophysics. Vol. 18, No 3, str. 257–267.
- Marković D., M. Jeremić, Č. Radenović (1996): *Savremena biofizika. 4. Zakasnela fluorescencija hlorofila*. Izdavač „Velarta”, Beograd, str. 1–105.
- Radenović, C., Jeremić, M., Marković, D., Kalauzi, A. (2003): *Tranzijenti indukcionog signala zakasnele fluorescencije hlorofila i njihovo kinetičko ponašanje*, Zbornik posvećen profesoru dr Dušanu Vučeliću, 123–134, Beograd.
- Radenović Č. (1992): *Proučavanje fotoindukovane bioluminescencije kod lista kukuruza*. Savremena poljoprivreda 40 (6) pp. 15–38.
- Radenović Č. (1994): *A study of delayed fluorescence in plant models: photosynthetic, transportation and membrane processes*. J. Serb. Chem. Soc. 59 (9): 595–617.
- Radenović Č., D. Marković, M. Jeremić (1994b): *Delayed chlorophyll fluorescence in plant models*. Photosynthetica 30 (1): pp. 1–24.
- Radenović Č., M. Jeremić, D. Fidler, D. Marković, Ž. Vučinić (1985): *A kinetic study of delayed fluorescence induction and its dependence on preceding darkness*. Period. biol. 87 (2): 304–306.
- Radenović Č., M. Somborac, eds. (2000): *Kukuruz na pragu trećeg milenijuma — sećanja, kazivanja i predviđanja*. Izdavač: Institut za kukuruz „Zemun Polje”, Beograd
- Radenović, Č. (2001): *The mechanism of oscillatory ion transport and biopotential across exited cell membrane*. Proceedings for natural sciences, Matica Srpska 100, 91–104, Novi Sad.
- Radenović, Č. & M. Jeremić (1996): *The study of delayed light emission in plant models*. Arch. biol. Sci. 48: 1–18.
- Radenović, Č. (1997): *Induction processes and activation energy of delayed chlorophyll fluorescence*. Proceedings for Natural Sciences of Matica Srpska 93: 5–14.
- Radenović, Č., A. Kalauzi, K. Konstantinov and G. Drinić (2007): *Dynamics of generating transients of delayed fluorescence induction signal and photosynthetic antennas: a possible relationship. Mathematical modeling approach*. Proc. Nat. Sci. Matica Srpska Novi Sad, No 112, 5–26

- Radenović, Č., Vučinić, Ž., Fidler, D., Penčić, M. (1981): *Contribution to the study of delayed light emission rhythms in the maize leaf (Zea mays L.)*, *Studia biophysica* 86: 143—147.
- Radenović, Č. (1985): *Boltzmanov izraz za aktivacionu energiju i njegova primena u biološkim sistemima*. Interna publikacija Instituta za kukuruz „Zemun Polje” Beograd, pp 1—9.
- Rubin, A. B., Focht, A. A., Venediktov, P. S. (1988): *Some kinetic properties of electron-transfer processes on the primary photosynthesis reaction*, *Transaction of the Moscow Society of Naturalists* 28:172—184.
- Strehler L. B., Arnold W. (1951): *Light production by green plants*. *Journal of Genetics and Physiology* 34, pp. 809—820.
- Trifunović V. (1986): *Četrdeset godina moderne selekcije kukuruza u Jugoslaviji*. In: „Genetika i oplemenjivanje kukuruza. Dostignuća i nove mogućnosti”, str. 5—46, Beograd
- Veselovski V. A., Veselova T. V. (1990): *Luminiscent Characteristic of Plants Photosynthetic Apparatus*. In: „Luminiscence of Plants”. Ed. Nauka, Moscow, pp. 8—78.
- Vučinić, Ž., B. Nešić & Č. Radenović (1982): *Delayed fluorescence as an in situ probe of fluidity changes in maize photosynthetic apparatus*. *Period. biol.* 84: 223—226.
- Раденович Ч., М. Еремич, Д. Маркович (1994а): *Фотоиндукционная биолуминесценция растительных систем: фотосинтетические, транспортные и мембранные процессы*. *Физиология и биохимия культурных растений* 26 (5): стр. 419—433.

МЕЋУЗАВИСНОСТ ОСЦИЛАЦИЈА И ТРАНЗИЈЕНАТА ИНДУКЦИОНИХ ПРОЦЕСА ЗАКАСНЕЛЕ ФЛУОРЕСЦЕНЦИЈЕ ХЛОРОФИЛА У ТИЛАКОИДНОЈ МЕМБРАНИ ИНТАКТНОГ ЛИСТА КУКУРУЗА — РЕАКЦИЈЕ НА ДЕЛОВАЊЕ ВИШИХ ТЕМПЕРАТУРА И СУШЕ

Чедомир Н. Раденовић^{1, 2}, Ксенија П. Марковић¹, Александар Р. Радојчић¹, Виолета Б. Анђелковић¹, Александар Ј. Калаузи³

¹ Институт за кукуруз „Земун Поље”, Београд, Србија

² Факултет за физичку хемију, Универзитет у Београду, Београд, Србија

³ Центар за мултидисциплинарне студије, Универзитет у Београду, Београд, Србија

Резиме

У овом раду остварује се примена побољшаног фотосинтетично-флуоресцентног метода у проучавању сложених индукционих процеса закаснеле флуоресценције (ЗФ) хлорофила, који су разложени на транзијенте: А, Б, Ц, Д и Е и код којих могу да настану осцилације. Међузависност осцилација и индукционих транзијената: А, Б, Ц, Д и Е јавља се код интактних листова линија (ЗПР 70ж и Oh 43) и хибрида (ЗПДЦ 360, ЗПСЦ 46А, ЗПСЦ 704 и ЗПСЦ 71) кукуруза који су гајени у стаклари у условима различите ваздушне суше, деловања виших температура и дефицита воде у подлози.

Посебно се указује на услове при којима долази до успостављања стандардних индукционих процеса 3Ф хлорофила. Даје се краћи опис стандардне индукционе криве 3Ф хлорофила. Знатно детаљније дају се експериментални резултати о разлагању индукционих процеса 3Ф хлорофила на транзијенте: А, Б, Ц, Д и Е. Показани су временски и физичко-хемијски параметри за проучавање индукционе транзијенте. Објашњени су њихова природа и механизми настајања.

Утврђене су карактеристике и механизам настајања осцилација индукционих процеса 3Ф хлорофила. Дати су услови при којима долази до појаве побуђеног стања, настајања флукуација и успостављања осцилација индукционих процеса 3Ф хлорофила у тилакоидној мембрани интактног листа линија и хибрида кукуруза. Изнета је хипотеза о механизму настајања осцилација. Објашњена је међузависност успостављања осцилација и транзијентата индукционих процеса 3Ф хлорофила.

*Zoran Ž. Tambur¹, Igor M. Stojanov²,
Sonja M. Konstantinović³, Dara V. Jovanović⁴,
Desanka Cenić-Milošević⁵, Dolores N. Opačić⁶*

¹ Institute for Hygiene, Military Medical Academy, Crnotravska 17, Belgrade

² Scientific Veterinary Institute, Rumenački put 20, Novi Sad

³ Institute for Orthopedic Surgery Hospital „Banjica”, Mihajla Avramovića 28, Belgrade

⁴ City Institute for Public Health, Bulevar Despota Stefana 54a, Belgrade

⁵ Faculty of Stomatology, Žarka Zrenjanina 179, Pančevo

⁶ Institute for Epidemiology, Military Medical Academy, Crnotravska 17, Belgrade

MULTI DRUG RESISTANCE OF *CAMPYLOBACTER JEJUNI* AND *CAMPYLOBACTER COLI* TO TESTED ANTIBIOTICS IN STRAINS ORIGINATING FROM HUMANS, POULTRY AND SWINE

ABSTRACT: Thermophilic *Campylobacter* are among the most common cause of bacterial enteritis in humans. Food animals are considered one of the most important sources of *Campylobacter* causing infections in man. *Campylobacter* infection is clinically mild and resolves spontaneously. In severe or long-lasting cases, treatment with antibiotics is necessary. Resistance of *Campylobacter* spp. to drugs used in treatment of infection is a matter of concern.

The aim of this paper is to determine presence of multi drug resistant strains of *Campylobacter jejuni* and *Campylobacter coli* isolated from animals and man.

Material for testing was obtained by scraping the cecum surface from boilers, pig cecum and colon, and human feces. For isolation *Campylobacter jejuni* and *Campylobacter coli* microaerophilic conditions, temperature of 42°C and antibiotic supplement were required to inhibit the growth of other intestinal bacteria. In this research, for sensitivity testing of *Campylobacter jejuni* and *Campylobacter coli* three different methods were used: disc diffusion test, E-test, and dilution agar method.

A total of 55 strains of *Campylobacter jejuni* and *Campylobacter coli*. Out of the total, 24 strains originated from man, 16 from broilers were isolated, and 15 from pigs. Multi-drug resistance was determined in cases when the strains were resistant to two or more antibiotics. Applying E-test, we detected that the largest number of *Campylobacter jejuni* were multi drug resistant to two antibiotics (41.2%), and three antibiotics (11.8%). Applying disc diffusion method it was detected that 5.9% of *Campylobacter jejuni* from man was resistant to four tested antibiotics. Applying all three methods, it was detected that the largest number of *Campylobacter strains* was resistant to two antibiotics and three antibiotics. Applying disc diffusion method it was detected that 50% of *Campylobacter coli* strains from pigs were resistant to three tested antibiotics.

KEY WORDS: *Campylobacter jejuni*, *Campylobacter coli*, antibiotics, multi drug resistance, humans, broilers, pigs

INTRODUCTION

The genus *Campylobacter* consists of 18 species, but humans are most often affected by thermophilic *Campylobacter*: *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari* and *Campylobacter upsaliensis*.

Thermophilic *Campylobacter* are extremely hard for cultivation since they require special media supplemented with antibiotics that suppress the growth of other enteric bacteria, microaerophilic atmosphere (5% O₂, 10% CO₂ and 85% N₂) and temperature of 42°C.

Animals are rarely infected, and if infected the disease is followed by mild symptoms (Lopez et al., 2002). *Campylobacter* colonize animals in different percentage. Poultry, aged two to three weeks, is colonized by thermophilic *Campylobacter* spp. 50—90% (Newell, 2002). Pigs are colonized in a smaller percentage than poultry.

Campylobacter jejuni and *Campylobacter coli* are the most common causes of enteric infection in humans worldwide. There are up to 400 000 000 cases per annum. An important factor for the occurrence of this disease is low infective doses of only 500 microorganisms (Walker et al., 1986).

Drugs of choice for treating campylobacteriosis are erythromycin, quinolone, tetracycline, ampicillin, chloramphenicol and gentamicin.

Nowadays there is a compelling evidence that there is an increases of resistance of bacteria genus *Campylobacter* to antibiotics administered in human treatment (Aarestrup and Engeborg, 2001, 2001; Aquinox et al., 2002; Avrain et al., 2003; Butzler, 2004; Bywater et al., 2004; Cardinale et al., 2002; Ge Beilei et al., 2002).

Campylobacter resistant strains have mainly emerged as a consequence of the use of antimicrobial agents in food animal production (Aarestrup and Engerberg, 2001; Savaşan et al., 2004).

The objective of this paper was to use different sensitivity methods and determine sensitivity of *Campylobacter* spp. (originating from humans, broilers and pigs) to erythromycin (E), tetracycline (TET), ampicillin (AM), chloramphenicol (CHL) and ciprofloxacin (CIP) and to determine percentage of multi drug resistance of *Campylobacter jejuni* and *Campylobacter coli*.

MATERIAL AND METHODS

The material for examination was obtained from broiler's cecum and pig colon, as well as human feces. For isolation of thermophilic *Campylobacter* standard methods were applied (Tambur, 2007). The following classical and commercial biochemical tests for identification of *Campylobacter* were used: catalase, oxidize, hydrolyze hippurate, hydrolyze indoxyl acetate, fast H₂S test, nalidixic acid resistance, and cephalothin resistance.

The final identification of *Campylobacter* spp. was done by automatic identification system API Campy (Bio Merieux, France). The obtained isolates were kept in brain and heart infusion broth (HIB) with 30% glycerol at the temperature of -70°C for further examination (Tambur, 2007).

For testing the sensitivity of *Campylobacter jejuni/coli* disc diffusion method according to Kirby-Bauer, agar dilution method and E-test were used.

In sensitivity testing of *Campylobacter* spp. disk diffusion method according to Kirby-Bauer Mueller Hinton agar was used, enriched with 5% sheep blood. Desired *Campylobacter* density was achieved by comparison to 0.5 MacFarland standard that is approximately close to the standard of 1×10^8 bacteria/ml. After incubation radius of inhibition growth for the examined antibiotics was measured and the strains were divided in S, I and R categories. These data were used for *Haemophilus* spp. and *Streptococcus pneumoniae*, based on the recommendation of Clinical Laboratory Standards Institute, SAD (CLSI) from 2006.

Minimal values of inhibitory concentration (MIC) for antibiotics were measured applying dilution method. According to standard (CLSI) for MH agar approximately 10^4 bacteria/ml were streaked, and this was achieved by applying 2 μ l of prepared inoculum of 10^7 bacteria/ml density.

Besides the examined strains, the referent strains were also streaked on agar plate (*Campylobacter jejuni* ATCC — 33560 and *Campylobacter coli* ATCC — 33559).

MIC was the lowest concentration of the examined antibiotics with no visible bacteria growth. Since there is no standard for limiting values in examining MIC for *Campylobacter*, minimal values for erythromycin were used that refer to *Streptococcus pneumoniae*, but for other antibiotics standards for *Enterobacteriaceae* were used (Miljković-Selimović et al., 2004).

E-test is a diffusion test for determining minimal inhibitory concentration (MIC) of antibiotic for particular bacteria. Single colonies of bacteria were suspended in 4 ml Mueller Hinton broth to obtain density suspension that is in accordance to McFarland scale equal to 0.5. The suspension of examined bacteria strains was streaked on Mueller Hinton agar with 5% of defibrinated sheep blood using sterile swab. E-test strips (AB Biodisk, Solna, Sweden), that consist of MIC reading scale in mg/ml and a two-letter code on the handle to designate the identity of the antibiotic, were applied on dried plates, for two minutes. The plates were incubated for 48 hours at 37°C microaerophilic, and the values of MIC were read. Minimal inhibitory concentration were read where the ellipse edge intersects the strip. The expected values were given by the producer.

For check of the aforementioned methods and quality of media referent strains of *Campylobacter jejuni* ATCC-33560 and *Campylobacter coli* — ATCC-33559 were used.

In the Table 1. the values of antibiotic concentration E-test strips are given, as well as expected values for result interpretation (Tambur, 2007).

Tab. 1 — A display of antibiotic concentration on E-test strips expressed in mg and MIC values for result interpretation

Antibiotics	Antibiotic concentration (µg)	Sensitivity (µg)	Intermediary sensitivity (µg)	Resistant (µg)
Ampicillin	0.016—256	≤ 8	16	≤ 32
Erythromycin	0.016—256	≤ 0.5	1—4	≤ 8
Chloramphenicol	0.016—256	≤ 8	16	≤ 32
Ciprofloxacin	0.002—32	≤ 1	2	≤ 4
Tetracycline	0.016—256	≤ 4	8	≤ 16

RESULTS

In Table 2 and 3 are displayed the results on presence of multi drug resistant strains of *Campylobacter jejuni* and *Campylobacter coli*. All the strains were resistant on 3 or more antibiotics, and were therefore found multi drug resistant. However, having in mind the importance of *Campylobacter* in human and veterinary medicine, and knowing that for treatment of this disease only 6 antibiotics may be used (5 were used in this testing) the tables also show the results that refer to the strains resistant only to 2 antibiotics (T a m b u r, 2007).

Tab. 2 — Multi drug resistant strains of *Campylobacter jejuni* originating from humans, broilers and pigs

Methods of <i>Campylobacter</i> sensitivity testing on antibiotics	Number of antibiotics that were resistant (in %)			
	2	3	4	5
<i>Disc diffusion method</i>				
Strains originating from humans	35.3	17.6	5.9	
Strains originating from pigs		20.0		
Strains originating from broilers	30.0		10.0	
<i>E-test</i>				
Strains originating from humans	41.2	11.8		
Strains originating from pigs	20.0	20.0		
Strains originating from broilers	30.0	10.0		
<i>Dilution method in agar</i>				
Strains originating from humans	47.1	11.8		
Strains originating from pigs	20.0	20.0		
Strains originating from broilers	30.0	10.0		

Three methods were used for sensitivity testing of thermophilic *Campylobacter* strains isolated from animals and man. For *Campylobacter jejuni* multi drug resistance to these antibiotics was detected. In most strains, there was a multidrug resistance to two antibiotics. The percent of this resistance was quite unified in the strains originating from humans, broilers and pigs. Only applying disc diffusion method with strains originating from humans and broilers, *Campylobacter jejuni* was multi drug resistant to four examined antibiotics.

Tab. 3 — The results of examining sensitivity of *Campylobacter coli* originating from humans, broilers and pigs to antibiotics

Method for sensitivity testing of <i>Campylobacters</i> on antibiotics	Number of antibiotics that were resistant (in %)			
	2	3	4	5
<i>Disc diffusion method</i>				
Strains originating from humans	42.9			
Strains originating from pigs	30.0	50.0		
Strains originating from broilers		16.7		
<i>E-test</i>				
Strains originating from humans	42.9			
Strains originating from pigs	30.0	40.0		
Strains originating from broilers		16.7		
<i>Dilution method in agar</i>				
Strains originating from humans	42.9			
Strains originating from pigs	40.0	40.0		
Strains originating from broilers		16.7		

Campylobacter coli was resistant when three methods were applied. Approximately the same resistance was found in two, but also three examined antibiotics. Different from the *Campylobacter jejuni* multi drug resistance in *Campylobacter* strains, originating from pigs and broilers was the same, but was considerably lower in the same strains originating from humans. Thermophilic *Campylobacter* were not multi drug resistant to four or five examined antibiotics.

DISCUSSION

Campylobacter is a zoonotic diseases. Although animals are colonized by *Campylobacter* in a great percent, they rarely become ill, yet present a reservoir of infection for humans. Man may become infected through consuming insufficiently thermically processed meat (Newel, 2002), consuming unpasteurized milk and contaminated water (Avrain et al., 2003) and in a contact with pets (Aquino et al., 2002).

Thermophilic *Campylobacter* most often cause enteric infection but may also cause extraenteric diseases. According to Butzler (2004), most frequent extraenteric forms of the disease are meningitis, endocarditis, septic arthritis, osteomyelitis and neonatal sepsis. There are several reports on myocarditis and complicated cases caused by *Campylobacter jejuni*.

In developed countries, the symptoms of intestinal *Campylobacter* are bloody stool, fever, abdominal colics. There are more frequent than in infection caused by *Salmonella* and *Shigella* spp. (Cooker et al., 2002).

Lesions in intestinal *Campylobacter* are expressed in inflammatory infiltrate in lamina propria and abscess of crypta similar to infection caused by bacteria of *Salmonella* and *Shigella*. Autopsy of patient with intestinal campylobacteriosis showed hemorrhagic necrosis of jejunum and ileum (Snelling et al., 2005).

According to a group of authors, secondary diseases, as consequences of primary infection caused by thermophilic *Campylobacter* spp., are Guillain-Barré syndrome (GBS) and Reiter syndrome (A v r a i n et al., 2003; B u t z - l e r, 2004).

Campylobacteriosis is a diseases of mild course and usually resolves spontaneously. In a patient with a heavy clinical course and prolonged course, treatment with antibiotics is suggested (A a r e s t r u p and E n g e b e r g, 2001). One of the drugs of choice is tetracycline.

Since the 80's of the last century, there has been a selection and resistant strains of thermophilic *Campylobacter* have spread in humans. This is due to uncontrolled use of antibiotics in veterinary medicine, especially quinolone and tetracycline (A a r e s t r u p and E n g e b e r g, 2001; B y w a t e r et al., 2004; C a r d i n a l e et al., 2002; S a v a ş a n et al., 2004).

Sensitivity testing by disc diffusion method detected that 35.3% of thermophilic *Campylobacter* originating from humans were resistant to two examined antibiotics, 17.6% to three and 5.9% to four antibiotics. Multiresistance in *Campylobacter* is not a rare case.

When examining sensitivity by E-test for thermophilic *Campylobacter* spp. originating from humans, it was detected that 41.2% was simultaneously resistant to two antibiotics, and in 11.8% to three antibiotics.

In testing sensitivity of thermophilic *Campylobacter* spp. originating from humans, using agar dilution method, it was detected that 47.1% were at the same time resistant to two examined antibiotics, 11.8% were resistant to three antibiotics. Multidrug resistance of *Campylobacter jejuni* (22%) was detected by H a k e n e n et al. (2003). In India thermophilic *Campylobacter* spp. were resistant in 30.6%, most often to ciprofloxacin, tetracycline and ciprofloxacin (J a i n et al., 2005).

Sensitivity testing by disc diffusion method showed that in 42.9% *Campylobacter coli*, originating from humans, was simultaneously resistant to two examined antibiotics. The obtained results of three methods coincide almost in all details. The same results were obtained in this research regarding the *Campylobacter* originating from broilers and pigs.

C a r d i n a l e et al. (2002) gave reports that in Senegal in 9.8% of thermophilic *Campylobacter*, originating from broilers, were multi drug resistant most often to nalidixic acid and ciprofloxacin. Multi drug resistance is frequent in *Campylobacter coli* strains. Multi drug resistance was noticed also in thermophilic *Campylobacter* originating from poultry in SAD. *Campylobacter coli*, 3.7% of the examined strains, were resistant to five tested antibiotics (G e B e i l e i et al., 2002).

CONCLUSION

Multi drug resistance on antibiotics was discovered in testing sensitivity of *Campylobacter jejuni* and *Campylobacter coli* originating from humans, broilers and pigs.

Percentage of multi drug resistant strains of *Campylobacter jejuni* were very similar in the strains originating from humans, broilers and pigs. *Campylobacter jejuni*, in the strains originating from humans and broilers, was detected in four tested antibiotics using disc diffusion test.

Multi drug resistance of *Campylobacter coli* originating from pigs and broilers were equal, but considerably lower in the strains originating from humans. No multi drug resistance of thermophilic *Campylobacter* was discovered on four or five tested antibiotics.

High percentage of resistant strains and multi drug resistant strains of *Campylobacter* to most frequently used antibiotics is explained by uncontrolled use of antibiotics in poultry and swine husbandry as a preventive measure and subdosage for growth promoter.

REFERENCES

- Aarestrup, M. Frank and Engberg, Jørgen (2001): *Antimicrobial resistance of thermophilic Campylobacter*, Vet. Res., 32, 311—321.
- Aquino, M. H. C., Pacheco, A. P. G., Ferreira, M. C. S. and Tibana, A. (2002): *Frequency of isolation and identification of thermophilic Campylobacter from animals in Brazil*, The Veterinary Journal, 164, 159—161.
- Avrain, Laetitia, Humbert, Florence, L'Hospitalier, Sanders Pascal, Vernozy-Rozand, Christine, Kempf, Isabelle (2003): *Antimicrobial resistance in Campylobacter from broilers: association with production type and antimicrobial use*, Veterinary Microbiology, 96, 267—276.
- Butzler, J. P. (2004): *Campylobacter, from obscurity to celebrity*, Clinical Microbiology and Infectious Diseases, 10, 868—876.
- Bywater, Robin, Deluyker, Hubert, Deroover, Erik, Jong de Anno, Marrion Hervé, McConville, Malcom et al. (2004): *A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals*, Journal of Antimicrobial Chemotherapy, 54, 744—754.
- Cardinale, E., Dromigny, J. A., Tall, F., Ndiaye, M., Konte, M., Perrier Gros-Claude, J. D. (2002): *Antimicrobial susceptibility of Campylobacter strains isolated from chicken carcasses in Senegal*, Revue Élev. Méd. vét. Pays trop., 55 (4), 259—264.
- Cooker, O. Akitovye, Iskpehi, D. Raphael, Thomas, N. Bolaji, Amisu O. Kehinde and Obi, C. Larry (2002): *Human Campylobacteriosis in developing countries*, Emerging Infectious Diseases, 8, 3, 237—243.
- Ge Beilei, Bodeis Sonya, Walker D. Robert, White G. David., Zhao Shaohua, McDermot F. Patrick, et al. (2002): *Comparison of the E-test and agar dilution for in vitro antimicrobial susceptibility testing of Campylobacter*, Journal of Antimicrobial Chemotherapy, 50, 487—494.
- Hakanen, Antti J., Lehtopolku, Mirva, Siitonen, Anja, Huovinen, Pentti and Kotilainen, Pirkko (2003): *Multidrug resistance in Campylobacter jejuni strains collected from Finnish patients during 1995—2000*, Journal of Antimicrobial Chemotherapy, 52, 1035—1039.

- Jain, Deepika, Sinha, Sushmita, Prasad, N. Kashi, Padney, M. Chandra (2005): *Campylobacter species and drug resistance in a north Indian rural community*, Transactions of the Royal Society of tropical Medicine and Hygiene, 99, 207—214.
- López, M. Clara, Giacoboni, Gabriela, Agostini, Adela, Cornero, J. Fernando, Tellechea, M. Diana, Trinidad, José Juan (2002): *Thermotolerant Campylobacters in domestic animals in a defined population in Buenos Aires, Argentina*, Preventive Veterinary Medicine, 55, 193—200.
- Miljković-Selimović, B., Mraović, M., Potkonjak, B., Babić, T., Kocić, B., Ristić, L. (2004): *Thermophilic Campylobacter resistance to five antimicrobial drugs*. Clinical Microbiology and Infection (14th European Congress of Clinical Microbiology and Infectious Diseases), vol 10 suppl 3, 108.
- Newel, G. Diane (2002): *The ecology of Campylobacter jejuni in avian and human hosts and in the environment*, International Journal of Infectious Disease, 6, 3516—3521.
- Savaşan, Serap, Çiftiçi, Alper, Diker, Serdar Kadir (2004): *Emergence of Quinolone resistance among chicken isolates of Campylobacter in Turkey*, Turk. J. Vet. Anim. Sci., 28, 391—397.
- Snelling, W. J., Matsuda, M., Moore, J. E. and Dooley, J. S. G. (2005): *Under the microscope Campylobacter jejuni*, Letters in Applied Microbiology, 41, 297—302.
- Tambur, Zoran (2007): *Ispitivanje osetljivosti termofilnih Campylobacter vrsta izolovanih od životinja i ljudi na atibiotike i hemioterapeutike*, fakultet Veterinarske medicine univerziteta u Beogradu, doktorska disertacija.
- Walker, I. Richard, Caldwell, M. Blake, Lee, C. Eileen, Guerry, Patricia, Trust, J. Trevor and Riuz-Palacios, M. Guillermo (1986): *Pathophysiology of Campylobacter enteritis*, Microbiological reviews, 50, 1, 81—94.

МУЛТИРЕЗИСТЕНЦИЈА *CAMPYLOBACTER JEJUNI* И *CAMPYLOBACTER COLI* НА ИСПИТИВАЊЕ АНТИБИОТИКЕ КОД СОЈЕВА ПОРЕКЛОМ ИЗ ЉУДИ, ЖИВИНЕ И СВИЊА

Зоран Ж. Тамбур¹, Игор М. Стојанов², Соња М. Константиновић³,
Дара В. Јовановић⁴, Десанка Ценић-Милошевић⁵, Долорес Н. Опачић⁶

¹ Институт за хигијену, Војно медицинска академија,
Црнотравска 17, Београд, Србија

² Научни Институт за ветеринарство Нови Сад, Руменачки пут 20, Србија

³ Институт за ортопедско-хируршке болести „Бањица”,
Михајла Аврамовића 28, Београд, Србија

⁴ Градски Завод за јавно здравље, Булевар Деспота Стефана 54а,
Београд, Србија

⁵ Стоматолошки факултет, Жарка Зрењанина 179, Панчево, Србија

⁶ Институт за епидемиологију, Војно медицинска академија,
Црнотравска 17, Београд, Србија

Резиме

Термофилне кампилобактерије су један од најчешћих узрочника бактеријских гастроентеритиса људи. Животиње су резервоар узрочника и са њих се *Campylobacter* врсте преносе на људе. Кампилобактериоза људи је болест благог тока која спонтано пролази. Код тежих облика обољења и пролонгиране болести неопходан је третман антибиотикима. У последње време забрињава појава резистенције кампилобактерија на антибиотике који се најчешће користе у клиничкој пракси.

Циљ рада је да се установи присуство мултирезистентних сојева *Campylobacter jejuni* и *Campylobacter coli* изолованих из животиња и људи.

За испитивање је коришћен материјал добијен стругањем површине цекума бројлера, цекума и колона свиња, као и фецеса људи. За изолацију сојева *Campylobacter jejuni* и *Campylobacter coli* било је неопходно обезбедити микроаерофилну атмосферу, температуру од 42°C и додати антибиотски суплемент ради спречавања раста осталих цревних бактерија. У оквиру овог истраживања изолованим сојевима *Campylobacter jejuni* и *Campylobacter coli* испитивана је осетљивост на антибиотике применом три методе: диск дифузионе, Е-теста и дилуционе методе у агару.

Укупно је изоловано 55 сојева *Campylobacter jejuni* и *Campylobacter coli*, 24 соја су била пореклом од људи, 16 пореклом од бројлера и 15 сојева пореклом из свиња. Мултирезистентним смо сматрали сојеве који су били резистентни на два и више антибиотика. Применом Е-теста установљен је код *Campylobacter jejuni* највећи број мултирезистентних сојева: на два антибиотика 41,2%, и на три антибиотика 11,8%. Код *Campylobacter jejuni* изолованих код људи диск дифузионим методом установљено је 5,9% сојева резистентних на четири испитивана антибиотика. Истим методом установљено је да је 50% сојева *Campylobacter coli* пореклом из свиња било резистентно на три испитивана антибиотика. Применом све три методе установљено је да је највећи број изолованих сојева *Campylobacter coli* мултирезистентан на два, односно три антибиотика.

*Janja P. Kuzevski¹, Nada A. Milošević²,
Saša B. Krstanović³, Zora S. Jeličić¹*

¹ Institute for science application in agriculture, Belgrade, Serbia

² Institute of field and vegetable crops, Novi Sad, Serbia

³ MK COMMERCE, DOO, Novi Sad, Serbia

EFFECT OF *AZOTOBACTER CHROOCOCCUM* ON SUGAR BEET AND MICROBIAL ACTIVITY OF RHIZOSPHERE

ABSTRACT: In sugar beet production, one of the most important factors that affect the yield, apart from genetic properties, is the use of mineral fertilizers. Considerate amounts of mineral fertilizers are used in sugar beet production. However, if agroecological conditions are not optimum, mineral fertilizers cannot be completely absorbed, which may lead to soil contamination. Therefore, research has been focusing on ways of using atmospheric nitrogen by means of nitrogen-fixing bacteria. Numerous researches have proved that one part of mineral fertilizers can be replaced by biological nitrogen.

The aim of this research was to determine the effect of genotype, azotobacter and the amount of mineral fertilizers on the root yield of sugar beet and on the microbiological activity of the sugar beet rhizospheric soil.

Three hybrids of sugar beet were used during the two years of the research. The seed of the hybrids was inoculated with three strains of azotobacter. Various amounts of NPK were used (0;30;60;90 kg/ha).

At the end of the vegetation period, the following were determined: root yield, total number of bacteria, number of azotobacter, oligotrophic bacteria, ammonifiers, fungi, and actinomycetes in soil. Dehydrogenase activity was measured.

The results were processed statistically (analysis of variance for factorial trials) and the effect of the factors was determined upon the expected mean square values.

The yield was mainly affected by the amount of mineral fertilizers. However, the effect of mineral fertilizers was different with different inoculation treatments. The effect of the examined factors was dependant upon genotype, amount of mineral fertilizers, inoculation and the year of trials. The interaction between genotype, mineral fertilizers, inoculation and the year of trials was the factor that had the greatest effect on the number of almost all the examined soil microorganisms.

KEY WORDS: sugar beet, root yield, *Azotobacter chroococcum*, microbiological activity of the soil

INTRODUCTION

The use of an adequate amount of mineral nutrients in sugar beet production is one of the basic prerequisites of high and stable yield. It is also one of

the best ways of exploiting the potential of the environment, as well as the genetic fertility potential of sugar beet. Mineral fertilizers provide the soil with easily accessible plant nutrients. The amount of mineral fertilizers to be used is determined upon the demands of the plant and the amount of nutrients in the soil. In agricultural production, large amounts of nitrogen fertilizers are used in order to achieve the highest possible yield.

However, modern agricultural production is concerned about the effects of mineral fertilizers on the environment. Mineral nitrogen is a useful source of nutrients for plants, but at the same time, a serious pollutant.

According to evidence from a great body of literature, it can be assumed that it is possible to replace one part of mineral nitrogen with biological nitrogen, which is plant-friendly. This, in turn, could prevent the pollution of the environment and lead to the production of food of higher ecological value. The research by Milošević et al. (1997) and Govedarica et al. (2002, 2004) proved that one part of mineral nitrogen could successfully be replaced with biological nitrogen fixed by diazotrophs (nitrogen-fixators). Therefore, a lot of attention is being given to the soil microorganisms, which can transform both the soil and atmospheric nitrogen into a form that is plant-friendly.

Mineral fertilizers are also a readily available source of nutrients for soil microorganisms themselves. The effect of mineral fertilizers on the microbiological activity depends on several factors: the amount and type of fertilizer, the type of soil, the plant and properties of microorganisms. In chernozem soil, for instance, the number of microorganisms and their activity were high, both with and without the application of mineral fertilizers (Govedarica and Jarak, 1995). In less fertile types of soil, the use of mineral fertilizers stimulated microbiological activity (Jarak et al., 1993).

Bearing in mind the importance of the rational use of nitrogen mineral fertilizers and a growing interest for ecologically sound agricultural production, the aim of this investigation was to determine the role of genotype and other factors that contribute to the root yield and affect the microbiological activity of sugar beet rhizospheric soil.

MATERIALS AND METHODS

Microexperiments were conducted for two years in fluvisol, on humogley, in the locality of Pančevački Rit.

The effect of seed inoculation with *Azotobacter chroococcum* on the root yield was investigated in three sugar beet hybrids (3n hybrids of the PKB Agroekonomik Institute).

The seed was inoculated with a liquid inoculum of *azotobacter* shortly before sowing. Three strains of *Azotobacter chroococcum* were used (strains 16, 84 and 86 of the Institute for Field and Vegetable Crops, Novi Sad and Faculty of Agriculture, Novi Sad). The control was not inoculated.

In order to determine the effect of mineral fertilizers and inoculation on the productivity of the three hybrids, there were several variants of fertilization (without NPK; N₃₀: P₃₀: K₃₀; N₆₀: P₆₀: K₆₀; N₉₀: P₉₀: K₉₀).

The experiments were performed by means of random block system in five repetitions. The plots' size was 13.5 m² in total, with the distance in a row being 0.20 m and the distance between rows 0.45 m.

All the necessary agrotechnical measures were applied properly and at the appropriate time.

At the end of the vegetation period, the root was taken out in order to observe its yield. At the same time, samples of the rhizospheric soil of sugar beet were taken in order to observe the microbiological activity. An average sample was prepared from the rhizosphere of fifty plants from the middle row of the plots. The samples included all repetitions and all variants (mineral fertilizers, inoculation) of every sugar beet genotype.

The total number of microorganisms (TN) in an agarized soil extract was determined by dissolution method. The number of ammonifiers (Am) was determined on MPA (Pochon and Tardieux, 1962). The number of free nitrogen-fixators was determined in Fjodorov medium, the number of oligotrophic bacteria (Olg) by dissolution method and the number of azotobacter (Azb) by fertile drops method. The number of actinomycetes (Act) was determined in a synthetic medium and the number of fungi (F) in Czapek medium. The fungi were grown at the temperature of 25°C and the other microorganisms at 28°C. The incubation period lasted two to seven days.

Dehydrogenase activity was determined by Lenhard's method (1956) and modified by Thalmann's method (1968). Dehydrogenase activity is shown in µg TPF per 1g of soil.

The obtained results were statistically analysed by the analysis of variance for factorial trials. The analysis concentrated on the effect of the main factors (the year of trials, sugar beet genotype, *Azotobacter chroococcum* genotype and mineral fertilizers) and their interaction. The effect of these on the root yield, number of microorganisms and dehydrogenase activity was measured by means of F test. The components of variances were determined upon the expected mean square values (Hadživuković, 1991).

RESULTS AND DISCUSSION

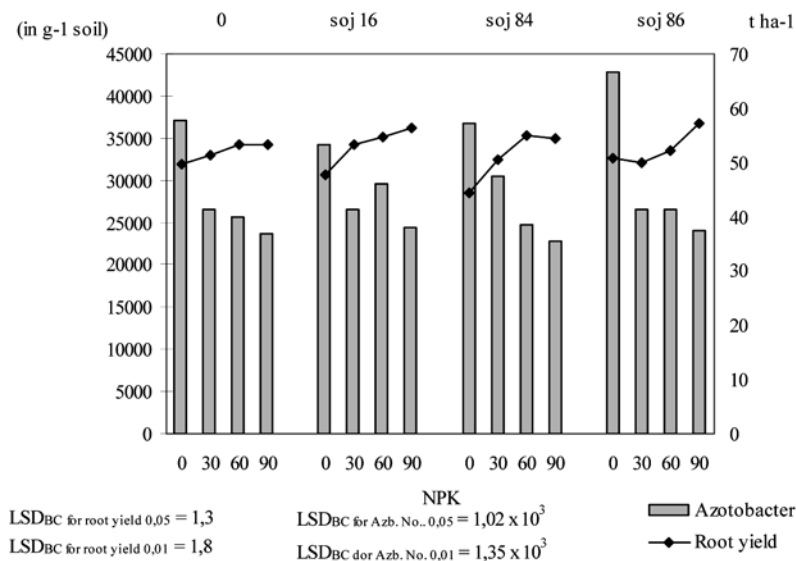
Soil microorganisms live in balanced relations, which are specific to each type of soil. Numerous agrotechnical measures frequently disrupt this balance. This affects the number of microorganisms and their enzymatic activity (Milošević et al., 1997; Milošević and Govedarica, 2002; Govedarica et al., 1999; Jarak et al., 1999).

Different amounts of mineral fertilizers that were used in this investigation, as well as the inoculation of the sugar beet seed with *Azotobacter chroococcum* strains had a considerable effect on the number of all the investigated microorganisms and dehydrogenase activity.

The amount of mineral fertilizers is an abiotic factor that may significantly affect the sugar beet root yield (Marinković et al., 1996). According to Stančić (1997) in the same level of mineral fertilizers, the most significant factor which affects the variation of the root yield is the genetic difference between the parent and the hybrid. According to Krstanović (2001), the root yield is primarily affected by environmental conditions. The root yield in our investigation was dependant upon the year of the trials, the amount of mineral fertilizers, sugar beet genotype and inoculation.

During the trials, numerous results were gathered. Graphs 1 and 2 present an average azotobacter number and dehydrogenase activity, as well as the root yield depending on the fertilizer applied. These indicators were chosen because the aim of the investigation was the effect of inoculation with *Azotobacter chroococcum* on the root yield, whereas dehydrogenase activity is an indicator of general biological activity of soil.

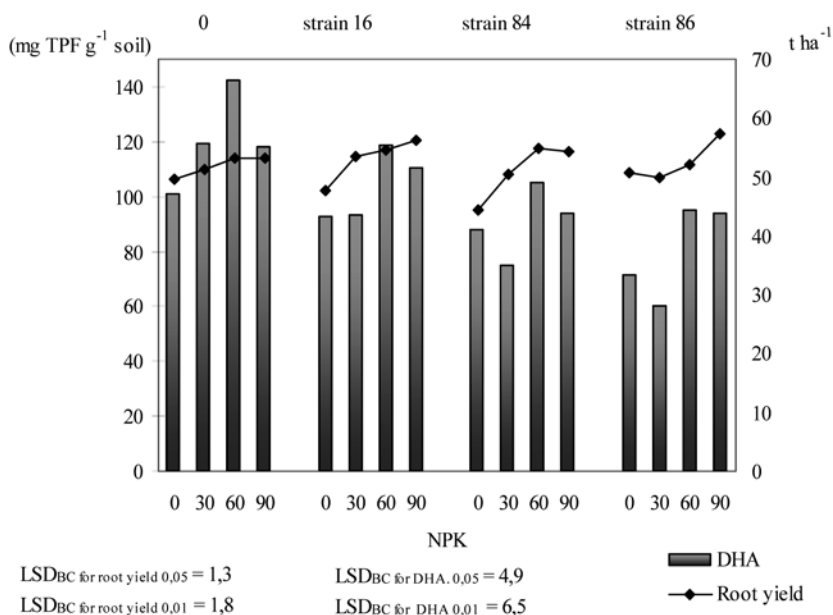
In all variants with inoculation, the greatest average number of azotobacters was observed when no mineral fertilizers were applied. Even earlier, papers by Milošević (1988) and Milošević et al. (1989) showed that high amounts of nitrogen fertilizers have a negative effect on the number of azotobacters in soil. Graph 1 also shows that the greatest number of azotobacter does not mean the highest average root yield.



Graph. 1 — The number of azotobacters in soil (g⁻¹ of soil) and the sugar beet root yield

Dehydrogenase activity and the root yield were highest in most variants with inoculation when 60kg ha⁻¹ NPK was applied. The exception was the variant with strain 86 and 90 kg ha⁻¹ NPK (Graph 2).

The obtained results showed that the number of the examined microorganisms and dehydrogenase activity in soil were highly affected by the following



Graph. 2 — Dehydrogenase activity in soil ($\mu\text{g TPF g}^{-1}$ of soil) and the sugar beet root yield

factors: the year of the trials, the amount of mineral fertilizers, inoculation, sugar beet genotype and their interactions (Table 1). However, the effect of the mentioned factors varied depending on the group of the examined microorganisms (Table 2).

It was observed that the year of the trials alone did not have a significant effect on the number of the most groups of microorganisms nor on the dehydrogenase activity. The number of microorganisms was primarily affected by the interaction of the genotype, mineral fertilizers, inoculation and the year of the trials. The year of the trials and mineral fertilizers/year interaction had a somewhat greater effect on the number of azotobacter in the soil. Dehydrogenase activity was primarily affected by the interaction between the mineral fertilizers and the year, and the interaction between the inoculation and the year of the trials (Table 2).

Tab. 1 — The analysis of variance of the microbiological activity of soil

Source of variation	df	Variance							F _{tab.}	
		TN*	Azb*	Olg*	Am*	F	Act*	DHA*	0.05	0.01
Repetitions	2	0.8	0.1	1.9	0.1	1.0	0.05	0.02	3.04	4.71
Sugar beet genotype (A)	2	27.4	3.5	11.2	98.5	420.5	8.0	2.4	3.04	4.71
Mineral fertilizer (B)	3	56.3	26.9	65.9	157.7	74.9	16.3	137.0	2.65	3.88
Inoculation(C)	3	96.9	0.4	65.3	455.8	83.2	56.3	211.5	2.65	3.88
Year (D)	1	895.0	82.3	161.2	146.0	170.1	17.3	25.9	3.89	6.76
Interaction AB	6	18.1	1.0	5.6	40.0	68.3	15.2	16.2	2.14	2.90
AC	6	61.9	2.3	93.1	73.1	380.0	6.0	41.4	2.14	2.90
AD	3	59.2	9.7	6.6	9.4	531.7	13.0	8.7	2.65	3.88
BC	9	73.1	1.2	12.7	46.3	297.4	10.8	9.9	1.92	2.50
BD	3	117.6	15.5	38.9	204.6	123.6	108.3	308.1	2.65	3.88
CD	3	240.8	2.1	142.8	173.4	515.9	32.7	325.1	2.65	3.88
ABC	18	17.6	1.2	20.8	52.5	290.9	13.0	15.0	1.65	2.03
ABD	6	61.8	0.6	10.9	18.8	276.8	6.8	3.8	2.14	2.90
ACD	6	3.5	3.1	100.5	149.7	213.3	9.1	22.0	2.14	2.90
BCD	9	96.8	0.9	40.1	61.4	617.9	9.6	9.2	1.92	2.50
ABCD	18	39.6	1.5	32.0	29.3	89.7	9.1	11.3	1.65	2.03
Error	192	0.2	0.02	2.9	0.2	2.9	0.1	0.006		

TN — total number
Am — ammonifiers
Azb — *Azotobacter*
F — fungi

Olg — oligotrophic bacteria
Act — *Actinomycetes*
DHA — dehydrogenase activity

* the value of the variance x 100

Tab. 2 — Variance components (%) for the number of microorganisms and dehydrogenase activity of soil

Source of variation	For the number of						DHA
	TN	Azb	Olg	Am	F	Act	
Sugar beet genotype (A)	0.0**	0.0**	0.0*	0.0**	0.0**	0.0**	0.0**
Mineral fertilizer (B)	0.0**	8.8**	1.0**	0.0**	0.0**	0.0**	0.0**
Inoculation (C)	0.0**	0.0**	0.0**	1.1**	0.0**	2.8**	0.0**
Year (D)	12.1**	21.7**	3.2**	0.0**	0.0**	0.0**	0.0**
Interaction AB	0.0**	0.0**	0.0	0.0**	0.0**	2.6**	0.8**
AC	3.0**	2.0**	9.7**	3.0**	6.5**	0.0**	5.3**
AD	1.3**	9.6**	0.0	0.0**	0.1**	0.8**	0.0**
BC	6.1**	0.0**	0.0**	1.6**	6.2**	0.1**	0.0**
BD	7.1**	21.7**	0.7**	9.1**	0.5**	27.9**	34.8**
CD	0.1**	0.5**	11.8**	7.5**	6.3**	27.6**	36.8**
ABC	0.0**	0.0**	0.0**	6.4**	18.0**	5.8**	2.6**
ABD	6.0**	0.0**	0.0**	0.0**	8.3**	0.0**	0.0**
ACD	0.0**	7.3**	21.8**	16.6**	5.5**	0.0**	3.7**
BCD	20.7**	0.0**	3.4**	5.9**	31.5**	0.6**	0.0**
ABCD	42.9**	27.0**	37.1**	48.4**	15.5**	30.3**	15.9**
Error	0.6	1.3	11.2	0.4	1.6	1.4	0.02
Total	100.00	100.0	100.0	100.0	100.0	100.0	100.0

TN — total number
Am — ammonifiers
Azb — *Azotobacter*
F — fungi

Olg — oligotrophic bacteria
Act — *Actinomycetes*
DHA — dehydrogenase activity

* F test 0,05
* F test 0,01

The analysis showed that the same yield was achieved both in the variant with strain 84 inoculation and 60 kg ha⁻¹, and in the control variant with 90 kg ha⁻¹ NPK. The effect of inoculation on the root yield was particularly obvious in the variant with strain 86 inoculation and 90 kg ha⁻¹ NPK. This combination was most effective in the whole experiment (Graph 1 and 2).

Mineral fertilizers also had a positive effect on the root yield of the sugar beet hybrids. The average root yield of the control increased significantly when a higher amount of NPK was applied. In inoculated variants, depending on the amount of NPK, the differences in the root yield of the investigated hybrids were much bigger (Graph 1 and Graph 2).

The F test pointed to the importance of all the investigated factors and their interactions (Table 3).

Tab. 3 — The effect of variance components (%) on the root yield of sugar beet

Source of variation	Variance components	
	σ^2	%
Year (A)	0	0.00**
Mineral fertilizers (B)	9710278	26.59**
Genotype (C)	0	0.00**
Inoculation (D)	0	0.00**
Interaction AB	845554	2.31**
AC	135566	0.37**
AD	144248	0.39**
BC	1368649	3.75**
BD	1065608	2.92**
CD	302943	0.83**
ABC	4351048	11.91**
ABD	1395673	3.82**
ACD	1802580	4.94**
BCD	2428473	6.65**
ABCD	4499112	12.32**
Error	8468320	23.89
Total	37610783	100.00

* F test 0.05

** F test 0.01

The analysis of variance of the results gathered during the two years showed that the amount of mineral fertilizers had the greatest effect on the phenotype variation of the sugar beet root yield. Different variants of inoculation had different effects on the root yield (Table 4).

In all inoculated variants, no significant effect of the genotype, the year or the interaction between the year and mineral fertilizers was observed. The interaction between the year and the genotype was significant only in the control variant (17.48%) whereas mineral fertilizers/genotype interaction was significant in the variant with strain 86 (26.59%). The effect of mineral fertilizers was most significant in the variants inoculated with strains 84 and 86 (Table 4).

Tab. 4 — The values of variance components

Source of variation	<i>Azotobacter</i> (strain)							
	0		strain 16		strain 84		strain 86	
	σ^2 (000)	%	σ^2 (000)	%	σ^2 (000)	%	σ^2 (000)	%
Year (A)	0*	0.00	0**	0.00	2946*	7.76	0	0.00
Mineral fertilizer (B)	291**	0.98	7037**	25.24	18160*	47.82	12851**	35.89
Genotype (C)	0	0.00	0**	0.00	0*	0.00	0	0.00
Interaction AB	1044**	3.53	1564**	5.61	0**	0.00	1075**	3.00
AC	5169**	17.48	0*	0.00	0	0.00	1687**	4.71
BC	0*	0.00	0**	0.00	777**	2.05	9522**	26.59
ABC	8944**	30.24	13416**	48.13	10402**	27.39	2679*	7.48
Error	14129	47.77	58580	21.01	5687	14.98	7998	22.33
Total	29577	100.00	27875	100.00	37973	100.00	35811	100.00

* F test 0.05

** F test 0.01

In all inoculated variants, the effect of the year/mineral fertilizer/genotype interaction was significant. The contribution of this interaction to the phenotype variation of the root yield ranged from 7.48% to 48.31% depending on the variant of inoculation (Table 4).

CONCLUSION

The number of almost all groups of microorganisms was primarily affected by the interaction of the year of the trials, mineral fertilizers, sugar beet genotype and inoculation.

The dehydrogenase activity was primarily affected by the interactions of the mineral fertilizers and the year, and by the inoculation and year of the trials.

The root yield was affected by the interaction of the year of the trials, mineral fertilizers, sugar beet genotype and inoculation.

By the mineral fertilizers, the root yield was affected at the full extent.

The mineral fertilizers affected significantly higher on the root yield in variants with the inoculations.

In order to determine the correlation between the root yield and the microbiological activity of soil, it would be necessary to examine all of the mentioned factors individually.

REFERENCE

- Hadživuković, S. (1991): *Statistički metodi s primenom u poljoprivrednim i biološkim istraživanjima*. Institut za ekonomiku poljoprivrede i sociologiju sela, Novi Sad, 584.
- Govedarica, M., Jarak, M. (1995): *Mikrobiologija zemljišta*. Univerzitet u Novom Sadu, Poljoprivredni fakultet. „Feljton”, Novi Sad, 189.

- Govedarica, M., Milošević, N., Jarak, M. (1999): *Uticaj sabijanja zemljišta na mikroorganizme i njihovu aktivnost*. Sabijanje zemljišta (Nikolić, R., ur.), Poljoprivredni fakultet, Novi Sad, 79—93.
- Govedarica, M., Milošević, N., Jarak, M., Đurić, S., Jeličić, Z., Kuzevski, J. (2002): *Primena biofertilizatora u proizvodnji kukuruza i šećerne repe*. Scientific papers, Faculty of agriculture, Timișoara, Romania, No XXXIV, 313—317.
- Govedarica, M., Jeličić, Z., Jarak, M., Milošević, N., Kuzevski, J., Krstanović, S. (2004): *Azotobacter chroococcum as alternative to conventional fertilization in the production of maize*. Zemljište i biljka, Vol. 55, No 3, 217—222.
- Jarak, M., Govedarica, M., Milošević, N., Vojvodić-Vuković, M., Klokocar-Smit, Z. (1993): *Effect of melioration practices on the microbiological characteristics of the solonetz soil*. Zemljište i biljka, Beograd, 42, 1, 9—14.
- Jarak, M., Govedarica, M., Milošević, N. (1999): *Mikroorganizmi i plodored. Plodoredi u ratarstvu* (Molnar, I., ur.), Naučni institut za ratarstvo i povrtarstvo, Novi Sad, 277—334.
- Krstanović, S. (2001): *Nasleđivanje kvantitativnih osobina korena šećerne repe*. Doktorska disertacija, Univerzitet u Beogradu, Poljoprivredni fakultet.
- Marinković, B., Crnoborac, J., Balešević, S. (1996): *Agrotehnički razlozi stagnacije i opadanja prinosa šećerne repe*. Zbornik radova, Naučni institut za ratarstvo i povrtarstvo, Novi Sad, 25, 129—136.
- Milošević, N., Govedarica, M., Jarak, M. (1997): *Mikrobi zemljišta: značaj i mogućnosti. Uređenje, korišćenje i očuvanje zemljišta*, Dragović, S. (ed), JDPZ, 389—398.
- Milošević, N., Kuzevski, J., Jarak, M., Govedarica, M., Krstanović, S. (1997): *Azotobakter-inokulaciona tehnologija u proizvodnji šećerne repe*. Zbornik naučnih radova PKB INI Agroekonomik, 3, 1, 109—119.
- Milošević, N. (1988): *Zastupljenost slobodnih diazotrofa u rizosferi šećerne repe*. Agronomski glasnik, 2/3, 3—12.
- Milošević, N., Jarak, M., Govedarica, M. (1989): *Dinamika ukupnog broja mikroorganizama i dehidrogenazna aktivnost u rizosferi šećerne repe u zavisnosti od NPK đubriva*. Agronomski glasnik, 4/5, 3—11.
- Milošević, N., Govedarica, M. (2002): *Effect of herbicides on microbiological properties of soil*. Zbornik Matice srpske za prirodne nauke, Novi Sad, 102, 5—21.
- Milošević, N. i Jarak, M. (2005): *Značaj azotofiksacije u snabdevanju biljaka azotom. U AZOT agrohemijski, agrotehnicki, fiziološki i ekoloski aspekti* (ur. R. Kastori), Naučni institut za ratarstvo i povrtarstvo, Novi Sad, 305—352.
- Pochon, J., Tardieux, P. (1962): *Techniques d'analyse en microbiologie du sol*. Ed. de la Turelle, Paris, 145.
- Stančić, I. (1997): *Genetička analiza kombinacionih sposobnosti roditeljskih genotipova i osobina F₁ generacije diploidnih hibrida šećerne repe*. Doktorska disertacija, Univerzitet u Beogradu, Poljoprivredni fakultet.
- Thalman, A. (1968): *Zur Methodik des Bestimmung des Dehydrogenase Aktivität im Boden mittels TTC*. Landw. Forsch., 21, 249—258.

УТИЦАЈ *AZOTOBACTER CHROOCOCCUM* НА УСЕВ И МИКРОБИОЛОШКУ АКТИВНОСТ РИЗОСФЕРЕ ШЕЋЕРНЕ РЕПЕ

Јања П. Кузевски¹, Нада А. Милошевић², Саша Б. Крстановић³,
Зора С. Јеличић¹

¹ Институт за примену науке у пољопривреди, Београд, Србија

² Институт за ратарство и повртарство, Нови Сад, Србија

³ МК COMMERCE, DOO, Нови Сад, Србија

Резиме

У производњи шећерне репе за постизање високих приноса, поред генетских својстава сорти, важан фактор је и примена минералног ђубрива. У овој производњи користе се значајне количине минералног ђубрива. Међутим, у случају да агроколошки услови нису оптимални за ваљано искоришћење унетог ђубрива оно неће бити искоришћено, те може утицати на загађење земљишта. Зато су већ дужи низ година истраживања усмерена у правцу изналажења могућности коришћења атмосферског азота посредством бактерија азотофиксатора. Многобројним испитивањима утврђено је да део минералног азота може бити замењен биолошким.

Циљ ових истраживања био је да се утврди удео генотипа шећерне репе, азотобактера и примењене количине минералног хранива на формирање приноса корена, као и на микробиолошку активност ризосфере шећерне репе.

У двогодишњим испитивањима утврђен је принос корена шећерне репе за три хибрида чије је семе непосредно пред сетву инокулисано азотобактером (три соја). У овим испитивањима примењене су различите количине NPK (0; 30; 60; 90 kg/ha активне материје NPK).

На крају вегетације утврђени су принос корена, као и укупан број бактерија, бројност азотобактера, олигонитрофила, амонификатора, гљива и актиномицета у земљишту. Такође је одређена дехидрогеназна активност земљишта.

Добијени подаци су статистички обрађени (анализа варијансе за факторијалне огледе), а удео чиниоца је одређен на основу очекиваних средина квадрата за факторијални оглед.

Највећи удео на варирање приноса корена имала је примењена количина минералног хранива. Међутим, њен удео значајно се разликовао зависно инокулације. Удео испитиваних фактора варирао је зависно од генотипа шећерне репе, примењене количине минералног хранива, инокулације и године испитивања.

Интеракција између генотипа шећерне репе, минералне исхране, инокулације и године испитивања имала је највећег удела у укупном варирању готово свих испитиваних група микроорганизама у земљишту.

Nataša P. Nikolić¹, Ljiljana S. Merkulov¹,
Borivoj Đ. Krstić¹, Slobodanka P. Pajević¹,
Milan K. Borišev¹, Saša S. Orlović²*

¹ University of Novi Sad, Faculty of Sciences and Mathematics,
Department of Biology and Ecology, Trg Dositeja Obradovića 2,
21000 Novi Sad, Serbia

² Institute of Lowland Forestry and Environment, Antona Čehova 13,
21000 Novi Sad, Serbia

* Author for correspondence. e-mail: natasa.nikolic@dbe.uns.ac.rs

VARIABILITY OF ACORN ANATOMICAL CHARACTERISTICS IN *QUERCUS ROBUR* L. GENOTYPES

ABSTRACT: The aim of this study was to examine variability of acorn anatomical characteristics in seventeen *Quercus robur* L. genotypes. Acorns were collected in clonal seed orchard Banov Brod (Srem, Vojvodina, Serbia). Microscopic measurements were done for pericarp (total thickness, thickness of exocarp and mesocarp), seed coat (total thickness, thickness of outer epidermis, parenchyma, and inner epidermis), and embryo axis (diameter, thickness of cortical region, and diameter of stellar zone). Obtained results revealed certain divergence between genotypes. The thickness of pericarp varied from 418 to 559 μm (genotypes 20 and 22, respectively). On average, the participation of exocarp in the total thickness of pericarp was 36.3%, of mesocarp 61.0%, while of endocarp 2.6%. The thickness of seed coat for individual genotypes ranged from 71 μm (genotype 28) to 157 μm (genotype 38). In addition, anatomic parameters of embryo axis varied among studied genotypes. The lowest cortical zone thickness and stellar zone diameter were measured in genotype 40, while the highest values in genotype 33.

KEY WORDS: acorn, anatomy, embryo axis, pericarp, seed coat, genotype variability, *Quercus robur*

INTRODUCTION

Oaks are among the most common tree species found throughout the world (Wittwer et al., 1993). Fruits of oaks, acorns, are botanically characterized as nuts. They are usually associated with an involucre forming a cup around the mature fruit. The acorn contains one seed with a straight embryo without endosperm (Bonner and Vozzo, 1987). During the acorn development, walls of the ovary are hardening into the pericarp, which surrounds the entire structure. Acorns are hard, one-seeded, dry, and indehiscent fruits (Holman and Robbins, 1944). In white oaks, including *Q. robur*, they

mature in the year of fertilization and are usually non-dormant. The acorn has a small embryo axis surrounded by well-developed cotyledons. After germination, the embryonic axis elongates to form the hypocotyl rudiments, while well-defined transitory region separates the axis centrally into cortical and stellar zones (Bonner and Vozzo, 1987).

Characteristics of individual parts of acorns are important in determination of seed viability, germination, and consequently establishment of young plants. Some species, including *Quercus robur*, have so-called recalcitrant seeds which do not naturally undergo significant drying and cannot survive desiccation (Roberts, 1973) to the low moisture contents required for prolonged storage (Greggains et al., 2000). These seeds are shed from the mother plant with high moisture content and thereafter remain sensitive to desiccation, losing viability as they lose water (Roberts, 1973). The pericarp appears to form the main natural barrier to desiccation under both natural and artificial conditions of seed storage (Sobrinho-Vesperinas and Viviani, 2000). Furthermore, it has been suggested that the pericarp of certain seeds contains substances that inhibit germination (Thapliyal and Naitani, 1996), and their presence may restrict gaseous exchange, water input, and embryo growth (Bradbeer, 1988; Miyajima, 1996). A thin seed coat adjoining the pericarp protects the embryo against mechanical injury and drying out. The role of the seed coat in controlling dormancy and germination is also known (Bewley and Black, 1994).

Variability of the seed anatomy among species is well documented. To our knowledge, variability of the acorn structure was not investigated within oak species. The aim of the present study was to examine variability of acorn anatomical characteristics in selected *Quercus robur* L. genotypes, bearing in mind their importance for preservation of seed viability.

MATERIAL AND METHODS

Acorns were sampled from 20-year-old *Q. robur* L. trees originating from the seed orchard Banov Brod (Srem, Serbia). The seed orchard was established by grafting of 85 English oak genotypes, seventeen of which were selected for this study: 4, 5, 6, 16, 18, 20, 21, 22, 25, 28, 29, 30, 33, 35, 38, 40, and 85. Acorns were collected from the ground after natural drop, and fixed in the mixture composed of ethyl alcohol and glycerol (v/v 50:50). Anatomical traits of pericarp and seed coat were studied at cross sections made in the middle part of the acorn, and the preparations were made using sliding microtome. Cross-sections of seeds at the level of embryo axis were made using freezing microtome. Microscopic measurements were done for pericarp (total thickness, thickness of exocarp and mesocarp), seed coat (total thickness, thickness of outer epidermis, parenchyma, and inner epidermis), and embryo axis (diameter, thickness of cortical region, and diameter of the stellar region). Three acorns were used for each genotype, of which 5 preparations were made (15 in total per genotype).

Participation of individual parts of the pericarp in its total thickness, as well as participation of cortical and stellar regions in the embryo axis diameter, was also calculated and expressed as percent.

The data were statistically processed using the analysis of variance (ANOVA). The genotypes were compared using the Duncan's test at $\alpha = 0.05$. Mean values of the studied parameters were ranked and marked with letters. Means followed with the same letter did not differ significantly at $p < 0.05$.

RESULTS

Pericarp

The pericarp is firm, and easily detached from the seed. It has three distinct layers visible on the cross sections: sclarified exocarp, multi-layered parenchymatous mesocarp, and a thin, one-layered endocarp (Figure 1).

The exocarp consisted of epidermis and mechanical cells. The epidermis is one-layered, composed of cells with thick walls. Several layers of mechanical cells (sclereids) leaning on the epidermis are followed with layers made up of parenchymatous cells (mesocarp). Cells of the internal layers of the mesocarp are flattened forming parenchymatous plates. There are relatively reduced vascular bundles in this part of the pericarp. The innermost part of the pericarp, endocarp, is composed of relatively small cells, and some of them form trichomes.

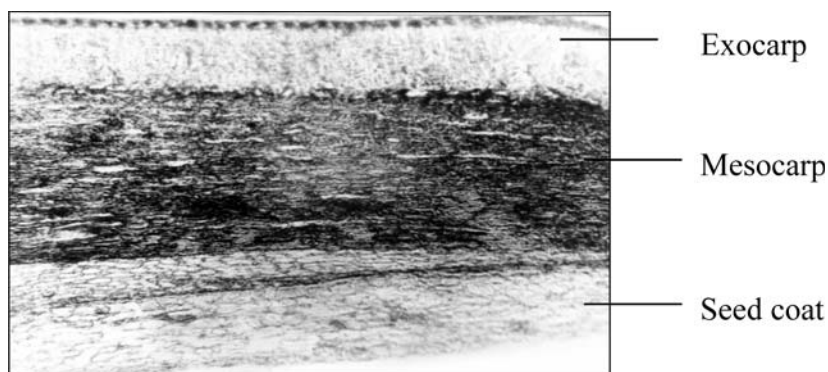


Fig. 1 — Cross section of pericarp (x100)

Measurements of anatomical parameters of the pericarp are summarized in Table 1. Slight variations in the total thickness of pericarp, exocarp, and mesocarp have been noticed. Thickness of pericarp varied from 418 (genotype 20) to 559 μm (genotype 22), with an average value of 503 μm for all genotypes. This trait also showed lower variability ($\text{CV} = 9.83\%$) than the thickness of exocarp ($\text{CV} = 10.43\%$) and mesocarp ($\text{CV} = 13.85\%$). The greatest thickness of exocarp was noticed in genotypes 6 and 30, and ranged from 240 (genotype 6) to 135 μm (genotype 38). On average, the thickness of this peri-

carp layer reached 182 μm . Thickness of mesocarp was the most variable anatomical trait of the pericarp, with the average value of 308 μm for all studied genotypes. The lowest value was recorded for genotype 20 (219 μm), and the highest for genotype 28 (382 μm). The lowest values of both mesocarp and pericarp thickness were noticed in genotype 20.

Tab. 1 — Anatomical characteristics of pericarp in *Q. robur* genotypes

Genotype	Thickness (μm)			
	Pericarp	Exocarp	Mesocarp	Endocarp
4	432 cd	181 bcd	238 ef	13
5	485 abcd	153 cde	319 abcde	13
6	537 ab	240 a	284 cdef	13
16	549 ab	188 bc	348 abcd	13
18	513 abcd	188 bc	312 abcde	13
20	418 d	186 bc	219 f	13
21	480 abcd	174 cd	293 bcdef	13
22	559 a	189 bc	357 abc	13
25	508 abcd	190 bc	305 abcde	13
28	555 ab	160 cde	382 a	13
29	425 d	166 cde	246 ef	13
30	507 abcd	225 a	269 def	13
33	526 abc	145 de	368 ab	13
35	459 bcd	183 bc	263 ef	13
38	505 abcd	135 e	357 abc	13
40	549 ab	217 ab	319 abcde	13
85	531 ab	167 cde	351 abc	13
<i>Average</i>	503	182	308	13
<i>LSD</i> _{0.05}	82.14	31.50	70.95	—
<i>CV</i> %	9.83	10.43	13.85	—

Participation of the exo-, meso- and endocarp in the pericarp total thickness varied between studied genotypes (Table 2), and average amounts were 36.3, 61.0, and 2.6%, respectively. All genotypes were characterized by the highest participation of mesocarp. The highest values of the exocarp participation, and the lowest of endocarp, were found in genotypes 6, 20 and 30. Among others, genotypes 28, 33, and 38 had the highest participation of mesocarp, and the lowest of exocarp, in the total thickness of pericarp.

Tab. 2 — Participation of individual parts of pericarp in its total thickness (%) in *Quercus robur* genotypes

Genotype	Exocarp	Mesocarp	Endocarp	ex/mz*
4	41.9	55.1	3.0	0.76
5	31.5	65.8	2.7	0.48
6	44.7	52.9	2.4	0.84
16	34.2	63.4	2.4	0.54
18	36.6	60.8	2.5	0.60
20	44.5	52.4	3.1	0.85
21	36.3	61.0	2.7	0.59
22	33.8	63.9	2.3	0.53
25	37.4	60.0	2.6	0.62
28	28.8	68.8	2.3	0.42
29	39.1	57.9	3.0	0.67
30	44.4	53.0	2.6	0.84
33	27.6	69.9	2.5	0.39
35	39.9	57.3	2.8	0.70
38	26.7	70.7	2.6	0.38
40	39.5	58.1	2.4	0.68
85	31.5	66.1	2.4	0.48
Average	36.4	61.01	2.61	0.60

* ex/mz — ratio between thickness of exocarp and thickness of mesocarp

Seed coat

A thin, brown seed coat is leaning to the inner side of the pericarp (Figure 1). It is made up of outer epidermis in which trichomes are formed, multi-layered parenchyma and inner epidermis leaning on cotyledons. Vascular bundles are placed in the parenchymatous layer. Individual cells of this layer contain crystals of calcium oxalate in the form of druses or crystal sand.

The thickness of seed coat was approximately five times smaller than the thickness of pericarp (Table 3). Values obtained for individual genotypes ranged from 71 (genotype 28) to 157 μm (genotype 38). Genotype 29 had the thickest outer epidermis (24.8 μm), while genotypes 18, 22, 28, 35, and 40 the thinnest (13 μm). The thickness of parenchyma varied between 45 (genotype 28) and 124 μm (genotype 38). Genotype 28 had the thinnest seed coat parenchyma and the thickest parenchymatous layer of the pericarp (mesocarp) (Tables 1 and 3). Of the studied seed coat traits, the thickness of parenchyma had the highest variability among the genotypes (CV = 16.32%), followed by the outer epidermis (CV = 13.71%). The genotypes did not differ in the thickness of inner epidermis.

Tab. 3 — Anatomical characteristics of seed coat in *Q. robur* genotypes

Genotype	Thickness (µm)			
	Seed coat	Outer epidermis	Parenchyma	Inner epidermis
4	144 abc	16.8 cd	114 ab	13.0
5	121 cde	14.7 cd	94 bcd	13.0
6	111 def	14.1 cd	84 cd	13.0
16	120 de	17.3 bc	90 cd	13.0
18	94 fgh	13.0 d	68 defg	13.0
20	108 def	14.1 cd	81 cde	13.0
21	128 bcd	14.7 cd	100 abc	13.0
22	83 gh	13.0 d	57 efg	13.0
25	113 def	17.1 bcd	83 cd	13.0
28	71 h	13.0 d	45 g	13.0
29	119 def	24.8 a	81 cde	13.0
30	102 efg	15.2 cd	74 def	13.0
33	81 gh	15.2 cd	52 fg	13.0
35	99 efg	13.0 d	73 def	13.0
38	157 a	20.8 b	124 a	13.0
40	82 gh	13.0 d	56 efg	13.0
85	147 ab	14.7 cd	120 a	13.0
<i>Average</i>	111	15.6	82	13.0
<i>LSD</i> _{0.05}	21.60	3.55	22.28	—
<i>CV</i> %	11.73	13.71	16.32	—

Embryo

The acorn contains one seed with a straight embryo (Figure 2) and well-developed, symmetrical cotyledons. Embryo consists of an axis, the hypocotyl — root axis, bearing at one end the root meristem, and at the other, the cotyledons and the meristem of the first shoot. Cortical and stellar zones as well as a continuous layer of the surface cells (protoderm) are distinguished in the embryo axis (Figure 3). A procambial system (the future vascular system), continuous throughout the hypocotyl and the cotyledons, is commonly differentiated in the embryo.

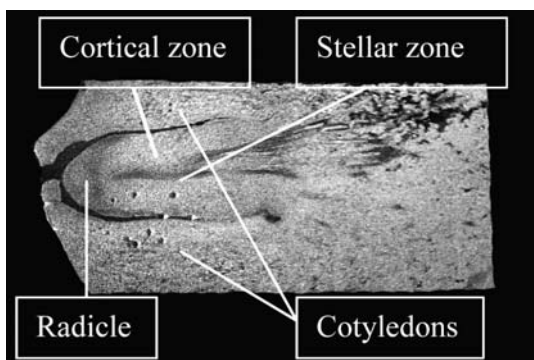


Fig. 2 — Median longitudinal section of embryo axis and cotyledons (x5)

Anatomical parameters of the embryo axis varied among studied genotypes (Table 4). The embryo axis diameter ranged between 1300 and 2000 μm . Thickness of the cortical zone varied from 378 to 584 μm , and diameter of the stellar zone from 544 to 898 μm .

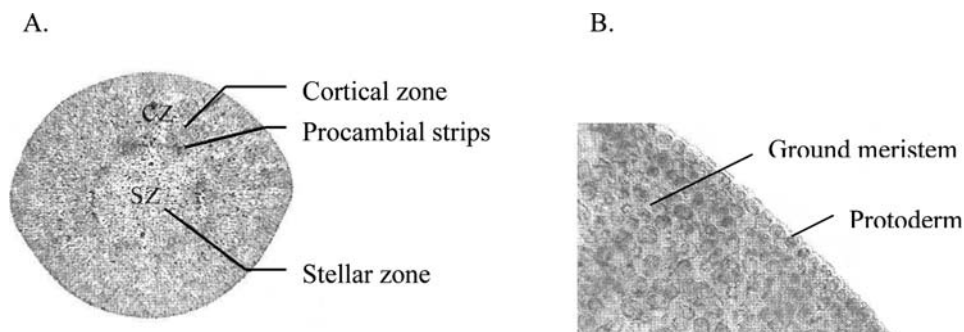


Fig. 3 — Cross section of embryo axis (A: x 100; B: x 400)

The greatest variability among studied genotypes was obtained for diameter of the embryo axis stellar zone (CV = 11.81%) and thickness of the embryo axis cortical zone (CV = 10.43%), followed by the diameter of the embryo axis (CV = 9.02%).

Tab. 4 — Dimensions of chosen structures of acorn embryo axis in *Q. robur* genotypes

Genotype	Diameter (μm)	Cortical zone thickness (μm)	Stellar zone diameter (μm)	d/cr* (%)	d/sr** (%)
4	1826 abc	464 bcd	898 a	50.82	49.18
5	1810 ab	504 b	802 ab	55.69	44.31
6	1710 bc	469 bcd	772 ab	54.85	45.15
16	1656 bc	431 bcd	794 ab	52.05	47.95
18	1766 abc	475 bc	816 ab	53.79	46.21
20	1518 bcd	395 cd	728 ab	52.04	47.96
21	1670 bc	476 bc	718 b	57.01	42.99
22	1582 bc	462 bcd	658 bc	58.41	41.59
25	1624 bc	451 bcd	722 b	55.54	44.46
28	1760 abc	504 ab	752 ab	57.27	42.73
29	1624 bc	457 bcd	710 b	56.28	43.72
30	1506 cd	424 bcd	658 bc	56.31	43.69
33	2000 a	584 a	832 ab	58.40	41.60
35	1594 bc	425 bcd	744 ab	53.32	46.68
38	1814 ab	498 ab	818 ab	54.91	45.09
40	1300 d	378 d	544 c	58.15	41.85
85	1676 bc	433 bcd	810 ab	51.67	48.33
Average	1584	461	752	55.09	44.91
LSD _{0.05}	125.4	79.9	73.8	—	—
CV%	9.02	10.43	11.81	—	—

* d/cr — allotment of the cortical region in embryo axis diameter (%)

** d/sr — allotment of the stellar region in embryo axis diameter (%)

Among others, genotype 33 had the highest diameter of the embryo axis, as well as the thickness of cortical zone. In contrast to it, genotype 40 had the lowest values of studied traits of the embryo. On average, studied traits of embryo axis amounted: the diameter — 1584 μm ; thickness of the cortical zone — 461 μm , diameter of the stellar zone — 752 μm .

On average, participation of the cortical zone in the diameter of the embryo axis was 55%, while of the stellar region 44.9% (Table 4). Genotypes differed by the participation of these two regions in the embryo axis diameter. Genotype 4 had the highest participation of the stellar, and the lowest of cortical zone, while genotypes 22, 30 and 40 had the highest participation of the cortical, and the lowest of stellar zone.

DISCUSSION

Variability of acorn structure between *Q. robur* genotypes was studied in this paper. Anatomical traits slightly differed between the genotypes as result of interaction between certain genotype and common environmental conditions.

The external surface of the ripe acorn presents two areas of different color, texture and composition (Sobrinho-Vesperinas and Viviani, 2000): cupular surface (corresponds to the area of attachment to the cupule), apical surface (area outside the cupule), and the transition band between them. The pericarp structure in the cupular region differs from that of the acorn apical region. The structure of the acorn cupular area is simple and homogenous, with vascular bundles composed of small, undifferentiated parenchymal cells having thick walls but lacking the cuticle or external cell layer (Sobrinho-Vesperinas and Viviani, 2000). Absence of the sclereids in this area enables the acorn growth (Zemkova, 1980). In the present work, structure of the pericarp in the apical acorn region was studied.

Structural characteristics and thickness of the pericarp are considered to have a major role in seed germination as they influence water absorption of the embryo, gas exchange, and leakage of inhibitors (Taylorson and Hendricks, 1977; Mohamed-Yasseen et al., 1994). Variability of the pericarp thickness between the genotypes was lower than of the seed coat. The thickest pericarp was found in genotype 22, along with genotypes 6, 16, 28, 40 and 85, while the thinnest in genotypes 20 and 29. However, other genotypes were characterized by the thickest (38, 85, 4) or the thinnest (28, 40, 33, 22, 18) seed coat. The mesocarp takes up 52—71% of the pericarp, and its thickness was the most variable trait of the pericarp. A negative correlation was found between the exocarp and mesocarp thickness, as well as between the pericarp and seed coat thickness ($r = -0.391$ and -0.393 , respectively), while the pericarp and mesocarp thickness were correlated positively ($r = 0.835$). Hence, well-protected acorns with firm pericarp may have thinner mesocarp (parenchyma). A well-developed parenchymatous tissue is important for accumulation of organic matters during the stages of acorn development. In addition, there are implications concerning the role of the parenchymatous layer (mesocarp) in absorption and retaining of water. Chestnut oak (*Q. prinus*)

acorns are much more capable to germinate in dry soils than acorns of white, black, or northern red oak (McQuilkin, 1990). This difference may be due to a thick parenchyma layer in the acorn pericarp that allows them to absorb and retain more moisture than acorns of other oaks (Korstian, 1927).

In the pericarp, thickness of sclerified exocarp ranged from 135 to 240 μm (genotypes 6 and 38, respectively). In almost all fruit types, the sclerotic cells are considered as a very important cellular type present in the pericarp, and they are related mainly to the protection of seeds from injuries, and the support of mesocarp fleshy tissues (Roth, 1977). Sobrino-Vespriñas and Viviani (2000) reported that the pericarp constitutes a barrier to water loss and protects recalcitrant seeds of *Q. suber* against desiccation. This barrier is functional both during acorn development and after shedding. *Q. robur* seeds are also recalcitrant, sensitive to desiccation, and they lose viability as they lose water (Roberts, 1973). Therefore, it could be assumed that acorns with thicker pericarp have better protection against desiccation, as found in genotypes 6, 16, 22, 28, 40 and 85. In some species, the pericarp of acorns is known to inhibit the natural germination process. In *Q. nigra* seeds, neither the pericarp nor the seed coat imposed significant barriers to passage of water. Therefore, Peterson (1983) assumed that delayed germination of *Q. nigra* seeds was caused by mechanical strength of the pericarp, chemical inhibition by the pericarp and slowly increasing capacity to imbibe water required for pericarp rupture. The pericarp structure changes during stratification and germination process. In stratified water oak acorns, Vozzo (1985) described pores in the exocarp with underlying highly branched tubular channels, which represent potential pathways for gas and water exchange.

The seed size is important for growth and development of plants, and is related to cotyledons as well as to embryo axis. A larger seeds potentially have more nutrient reserves stored in the cotyledons to feed the radicle and rapidly growing root system. The seed is still supporting the root growth long after the root has started to branch. The increase of plants during growth is based on their present size because of the geometric rate of cell division and therefore a larger seed produces more cells faster (Mottz, 1996).

During germination, carbohydrate and lipid contents in embryo diminish and both substances shift from the cotyledons to the embryo axis. Unlike carbohydrates, proteins were found near the shoot apex and not in the cotyledons (Vozzo and Young, 1975). Previous studies have shown that larger acorns produce bigger plants faster than smaller ones, due to more cotyledon tissue to feed the radicle and rapidly growing root system (Tecklin and McCreary, 1990). Dimensions of the embryo axis diameter and its parts, cortical and stellar region, are important for development of the future plants considering different tissues that will be formed. The protoderm become the epidermal tissue, cells of the ground meristem differentiate into a cortex, while the procambium become the vascular tissue. The ground meristem is distinguished from the procambium by its less elongated and wider cells, but both kinds of cells are filled with storage products in the dormant embryo (Paulson and Srivastava, 1968). Development of the procambium in the stellar region is of particular significance considering the role of the future conducting system

in the transport of matters necessary for plant development. Diameter of the stellar zone showed the highest genotypic variability (CV = 11.8%) of all tested embryo axis traits.

CONCLUSION

Genotype specificity of anatomical traits of acorns was evident in studied *Q. robur* genotypes. The thinnest pericarp and a very thin cortical zone of the embryo were found in genotype 20. The lowest cortical zone thickness and stellar zone diameter were measured in genotype 40, while the highest values in genotype 33. Analyses of traits of the pericarp and seed in different genotypes can be of significance in determination of seed quality necessary for programs related to improvement, breeding, selection, propagation, and seed storage in English oak, bearing in mind their importance for preservation of seed viability.

REFERENCES

- Bewley, J. D., Black, M. (1994): *Seeds: Physiology of Development and Germination*, Second Edition, Plenum Press, New York, 367 pp.
- Bonner, F. T., Vozzo, J. A. (1987): *Seed biology and technology of Quercus*, General technical report SO-66, US Department of Agriculture, Forest Service, Southern Forest Experiment Station, New Orleans, LA, 21 p.
- Bradbeer, J. W. (1988): *Seed Dormancy and Germination*, Blackie, Glasgow.
- Greggains, V., Finch-Savage, W. E., Quick, W. P., Atherton, N. M. (2000): *Putative desiccation tolerance mechanisms in orthodox and recalcitrant seeds of the genus Acer*, Seed Sci. Res., 10, 317—327.
- Holman, R. M., Robbins, W. A. (1944): *A Textbook of General Botany*, Wiley and Sons Inc., New York, 664 p.
- Korstian, C. F. (1927): *Factors controlling germination and early survival in oaks*, Bulletin No. 19, Yale University School of Forestry, New Haven, CT, 115 p.
- Leadem, C. L., Gillies, S. L., Yearseley, H. K., Sit, V., Spittlehouse, D. L., Burton, P. J. (1997): *Field Studies of Seed Biology*, Land Management Handbook. Available at <http://www.for.gov.bc.ca/hfd/pubs/Docs/Lmh/Lmh40.htm>.
- McQuilkin, R. A. (1990): *Quercus prinus L. chestnut oak*. In: Burns, R. M., Honkala, B. H. (tech. coords.) *Silvics of North America, Vol. 2: Hardwoods*, Agriculture Handbook 654, U.S. Department of Agriculture, Forest Service, Washington, DC, 726 p.
- Miyajima, D. (1996): *Germination of zinnia seed with and without pericarp*, Seed Sci. Technol., 24, 465—473.
- Mohamed-Yasseen, Y., Barringer, S. A., Splittstoesser, W. A., Costanza, S. (1994): *The role of seed coat in seed viability*, Bot. Rev., 60, 426—439.
- Motz, R. W. (1996): *Acorn collection, storage, sorting, and planting for the establishment of native oaks without supplemental irrigation*, Proceedings of a Symposium on Oak Woodlands: Ecology, Management, and Urban Interface Issues, March 19—22, San Luis Obispo, California, 678—683.

- Paulson, R. E., Srivastava, L. M. (1968): *The fine structure of the embryo of Lactuca sativa. I. Dry embryo*, Can. J. Bot., 46, 1437—1445.
- Peterson, J. K. (1983): *Mechanisms involved in delayed germination of Quercus nigra L. seeds*, Ann. Bot., 52, 81—92.
- Roberts, E. H. (1973): *Predicting the storage life of seeds*, Seed Sci. Technol., 1, 499—514.
- Roth, I. (1977): *Fruits of Angiosperms*, Gebrüder Borntraeger, Berlin.
- Sobrinho-Vesperinas, E., Viviani, A. B. (2000): *Pericarp micromorphology and dehydration characteristics of Quercus suber acorns*, Seed Sci. Res., 10, 401—407.
- Taylorson, R. B., Hendricks, S. B. (1977): *Dormancy in seeds*, Annu. Rev. Plant Physiol., 28, 331—354.
- Tecklin, J., McCreary, D. (1990): *Acorn size as a factor in early seedling growth of blue oaks*, Paper presented at the Symposium on Oak Woodlands and Hardwood Rangeland Management, October 31—November 2, Davis, California.
- Thapliyal, R. C., Naithani, K. C. (1996): *Inhibition of germination in Nyctanthes arborescens (Olacaceae) by pericarp*, Seed Sci. Technol., 24, 67—73.
- Vozzo, J. A. (1985): *Pericarp changes observed during Quercus nigra germination*, Seed Sci. Technol., 13, 1—9.
- Vozzo, J. A., Young, R. W. (1975): *Carbohydrate, lipid, and protein distribution in dormant, stratified, and germinated Quercus nigra embryos*, Bot. Gaz., 136 (3), 306—311.
- Wittwer, R. F., Barden, C. J., Anderson, S. (1993): *Growing oak trees from seed*, Oklahoma Cooperative Extension Service, Division of Agricultural Sciences and Natural Resources, Extension Facts F-5031, not paginated. Available at www.agweb.okstate.edu/pearl/forestry/general/f-5031.pdf.
- Zemkova, R. I. (1980): *Vrediteli generativnih organov listvenih introducentov*, Naukova dumka, Kiev, pp. 199.

ВАРИЈАБИЛНОСТ АНАТОМСКИХ ОСОБИНА ЖИРА КОД ГЕНОТИПОВА ХРАСТА ЛУЖЊАКА (*QUERCUS ROBUR* L.)

Наташа П. Николић¹, Љиљана С. Меркулов¹, Боривој Ђ. Крстић¹,
Слободанка П. Пајевић¹, Милан К. Боришев¹, Саша С. Орловић²

¹ Универзитет у Новом Саду, ПМФ, Департман за биологију и екологију,
Трг Доситеја Обрадовића 2, 21000 Нови Сад, Република Србија

² Институт за низијско шумарство и животну средину, Антона Чехова 13,
21000 Нови Сад, Република Србија

Резиме

У раду је испитана варијабилност анатомских особина жири код седамнаест генотипова храста лужњака (*Quercus robur* L.). Жир је сакупљен у вегетативној семенској плантажи Банов Брод (Срем, Војводина, Србија). Испитиване су структурне особине перикарпа (дебљина егзокарпа, мезокарпа и ендокарпа, као и укупна дебелина), семењаче (укупна дебелина, као и дебелина спољашњег епидермиса, паренхима и унутрашњег епидермиса) и ембрионове осе (дијаметар, де-

бљина кортикалне зоне и дијаметар стеларне зоне). Добијени резултати указују на одређену дивергентност генотипова у погледу проучаваних својстава. Дебљина перикарпа се кретала од 418 до 559 μm (генотипови 20 и 22). У просеку, удео егзокарпа у укупној дебљини перикарпа износио је 36.3%, мезокарпа 61.0%, а ендокарпа 2.6%. Дебљина семењаче код појединих генотипова варираше од 71 (генотип 28) до 157 μm (генотип 38). Такође, анатомске карактеристике ембрионске осе варираше су између генотипова. Најтања кортикална зона и најмањи дијаметар стеларне зоне утврђени су код генотипа 40, док су највише вредности ових параметара утврђене код генотипа 33.

*Ivana J. Sedej, Marijana B. Sakač,
Aleksandra Č. Mišan, Anamarija I. Mandić*

Institute for Food Technology, University of Novi Sad,
Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

ANTIOXIDANT ACTIVITY OF WHEAT AND BUCKWHEAT FLOURS

ABSTRACT: Antioxidative activities of wheat flours (type 500 and wholegrain) and buckwheat flours (light and wholegrain) were tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH[•])-scavenging activity, reducing power and chelating activity on Fe²⁺. Also, the content of the total phenolics of ethanolic extracts was estimated.

Polyphenolics content (expressed as gallic acid equivalent, GAE) in wheat flours varied between 37.1 and 137.2 µg GAE/g extract, while its content in buckwheat flour were at least four time higher and ranged between 476.3 and 618.9 µg GAE/g extract.

Ethanolic extracts of buckwheat flours exhibited higher antioxidant activities in all the assays, except for chelating activity.

Regarding all the obtained results, it can be concluded that bakery products produced with buckwheat flour could be regarded as potential functional foods.

KEY WORDS: antioxidant activity, polyphenolics, wheat and buckwheat flours

INTRODUCTION

Buckwheat, unlike most cereals, is an alternative crop belonging to the Polygonaceae family. The increasing attention for buckwheat cultivation and utilisation of buckwheat products is due to rising number of data focused on its functional characteristics, which can provide many health benefits based on buckwheat products consumption, first of all during prevention and healing chronic diseases (L i and Z h a n g, 2001).

Functional properties of buckwheat based foods are due to proteins and many rare components with healing effects. Among them, the most attractive ones are flavones, flavonoids, phytosterols, fagophyrins, and thiamin-binding proteins found in buckwheat seed.

C o d y and co-workers (1986) reviewed the biological and pharmaceutical effects of plant flavonoids, including buckwheat flavonoids, on human beings and test animals. Many of the biological functions, such as antimutagenicity, anticarcinogenicity, and antiaging, originate from antioxidant activity

of antioxidative enzymes and nonenzymic antioxidants (Cook and Samman, 1996). Cody and co-workers (1986) cited the medical effects of plant flavonoids; they are known for their effectiveness in reducing cholesterol levels in the blood, keeping capillaries and arteries strong and flexible, and assisting as a preventative measure against blood pressure, as well as many other cardiovascular diseases.

Omah and Mazza (1996) reported that flavonoids in buckwheat can perform high antioxidative activity that may have the potential to show pharmaceutical effects from this characteristic.

Six flavonoids (rutin, orientin, vitexin, quercetin, isovitexin and isoorientin) have been isolated and identified in buckwheat, but in buckwheat seed only rutin and isovitexin were found, and rutin attributed most of the flavonoid content in buckwheat seed (Dietrich-Szostak and Oleszek, 1999). Rutin and its hemisynthetic derivatives exert different medical effects like normalisation of increased vascular permeability and fragility, oedema protection (Ihme et al., 1996), antioxidant (Wojcicki et al., 1995), hypotensive (Evans, 1996), and antiinflammatory effects.

Flavonoids isolated from buckwheat hulls showed radical scavenging activity (Watanabe et al., 1997; Watanabe, 1998), which is important in suppressing radical damage in lipid peroxidation processes involved in food deterioration or some diseases. Holasova and co-workers (2002) also reported that phenolic compounds in buckwheat, namely 3-flavanols, rutin, phenolic acids and their derivatives, possessed antioxidative activity stronger than antioxidative components of oats and barley.

Based on the fact that antioxidative components from buckwheat flour significantly contribute to its functionality, the aim of this work was to investigate antioxidative properties of the commercially accessible buckwheat flours in comparison to the wheat flour type 500 and wholegrain wheat flour, the most frequently used wheat products for bakery industry, by measuring DPPH radical scavenging activity, reducing power, chelating effect on Fe^{2+} and total phenolics content.

MATERIALS AND METHODS

Materials

Buckwheat flours (light and wholegrain) and wheat flours (type 500 and wholegrain) were provided by local market.

Extraction

Buckwheat or wheat flour (10 g) was mixed with 100 mL of 96% ethanol. Extraction was carried out with shaking at room temperature during 1 h. Extract was separated by filtering through the filter paper (Whatman, Grade 4 Chr, UK), and procedure was repeated with 100 mL of solvent two times. The

extraction solutions (3 x 100 mL) were combined and dried by vacuum-evaporator. The dried extract was weight and the yield was calculated based on the wet weight of the sample. The dried extract was resolved in 96% ethanol to obtained 10 mL volume. The extract obtained by this procedure was used for further investigations of antioxidant activity.

Determination of total phenolics content

The total phenolics content in investigated extracts of buckwheat and wheat flours, measured as gallic acid equivalents, were determined spectrophotometrically using Folin-Ciocalteu's reagent (Singleton et al., 1999). The extract (0.1 mL) of buckwheat or wheat flours was diluted with distilled water (7.9 mL). Folin-Ciocalteu's reagent (0.5 mL) and sodium carbonate solution (1.5 mL; concentration 20 g/100 mL) were added, and the reaction mixture was mixed thoroughly. The mixture was allowed to stand for 120 min with intermittent shaking. The absorbance was determined in a spectrophotometer Jenway (6405 UV/Vis) at 750 nm.

DPPH free radical-scavenging activity test

The effect of the examined extracts on the content of 1,1-diphenyl-2-picrylhydrazyl (DPPH[•]) radicals was estimated according to the modified method of Hatano and co-workers (1988). The concentration of the DPPH[•] solution which was used in the assay was 90 μ M (22.5 mL 0.4 mM DPPH[•] solution (0.01577 g DPPH[•] in 100 mL methanol) was diluted with 95% methanol to 100 mL). An aliquot (1.0 mL) of the DPPH[•] solution (90 μ M) was diluted in 2.9 mL methanol, and 0.1 mL of the examined extracts at various concentrations (0.1, 0.5, 1.0 and 2.0 mg/mL for buckwheat flour extracts and 10, 20, 30 and 40 mg/mL for wheat flour extracts) was added. The mixture was shaken vigorously and left to stand for 60 min in the dark, then the absorbance was measured at 517 nm against the blank (without extract) in a Jenway (6405 UV/Vis) spectrophotometer.

IC₅₀ (mg/mL) was defined as the concentration of an antioxidant extract which was required to quench 50% of the initial DPPH[•] under the experimental conditions given. It was obtained by interpolation from linear regression analysis.

BHT and α -tocopherol were used as controls.

Reducing power

The reducing power of the ethanolic extracts was measured according to the method of Oyaizu (1986). Various concentrations (0.1, 0.5, 1.0 and 2.0 mg/mL for buckwheat flour extracts and 10, 20, 30 and 40 mg/mL for wheat flour extracts) of the ethanolic extracts (0.5 mL) were mixed with 2.5 mL of

phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixtures were incubated at 50°C for 20 min, and after that TCA (10%, 2.5 mL) was added. The mixtures were centrifuged at 650 g for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride and the absorbance was measured at 700 nm in a Jenway (6405 UV/Vis) spectrophotometer. Higher absorbance of the reaction mixture indicates greater reducing power.

IC₅₀ value (mg/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis.

BHT was used as control.

Chelating activity on Fe²⁺

The chelating activity of the ethanolic extracts on Fe²⁺ was measured according to the method of Decker and Welch (1990). Aliquots of 1 mL of different concentrations of ethanolic extracts of buckwheat and wheat flours (0.1, 0.5, 1.0 and 2.0 mg/mL and 0.01, 0.05, 0.1 and 0.5 mg/mL, respectively) were mixed with 3.7 mL deionized water. The mixture was left for reaction with FeSO₄ (2 mM, 0.1 mL) and ferrozine (5 mM, 0.2 mL) for 10 min at room temperature, and then the absorbance was measured at 562 nm in a Jenway (6405 UV/Vis) spectrophotometer. A lower absorbance indicates a higher chelating power.

The chelating activity on Fe²⁺ of the ethanolic extracts was compared with that of EDTA at a level of 0.036 mM.

Statistical analysis

Experimental results were given as mean ± SD of three parallel trials and measurements. *P* values < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

The total phenolic content of each flour extract was estimated, since phenolics may significantly contribute to its overall antioxidant activity. The amount of total phenolics in wheat and buckwheat flours expressed as µg of gallic acid equivalents (GAE) per 1 g of extract was presented in Figure 1. Phenolics content in wheat flours varied between 37.1 and 137.2 µg GAE/g extract, while its content in buckwheat flours was at least forty-five time higher and ranged between 476.3 and 618.9 µg GAE/g extract.

Comparing the results among flour types, higher content of phenolics was found in wholegrain flour in both, wheat and buckwheat. Results with similar trend of increased content of phenolics in the flours containing more outer

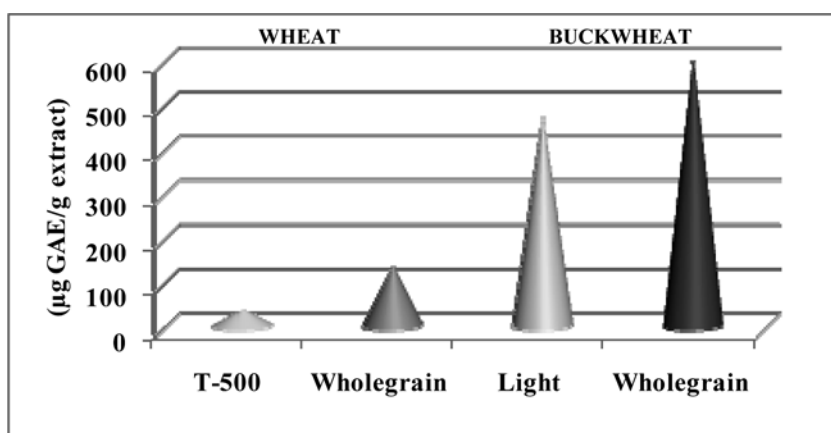


Fig. 1 — Total phenolic content of flours

layers of grain and bran were obtained in the study of Hung and Morita (2008).

The investigated extracts differed significantly ($P < 0.05$) in their total phenolics content that is contributed to the different abilities to inhibit lipid peroxidation (Fig. 2—4), i.e. to exhibited differences in antioxidative activities (AOA). Zielinski and Kozłowska (2000) have the statistically significant correlation between antioxidative activities and total phenolics of cereals and their fractions. A correlation between antioxidative activity and rutin content or total flavonoids content in buckwheat cultivars has been shown (Jiang et al., 2006).

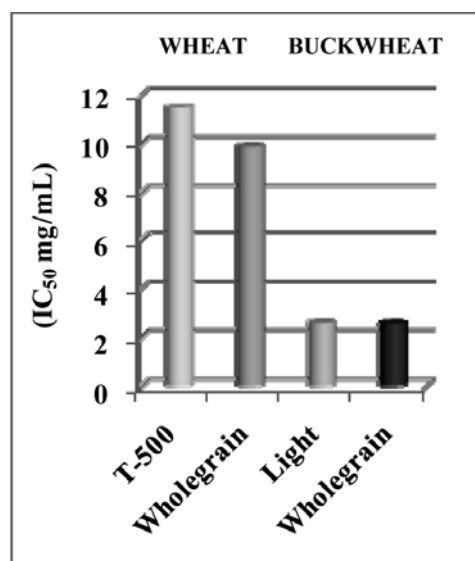


Fig. 2 — Reducing power

High differences in AOA comparing wheat and buckwheat flour extracts were showed in reducing power assay (Figure 2). Better antioxidant activity was found in buckwheat than in wheat flours indicated with lower IC_{50} values.

Strong antioxidative activity of buckwheat flour extracts might be attributed to the presence of polyphenols, especially rutin, as the main antioxidative component in buckwheat (Dietrych-Szostak and Oleszek, 1999). Rutin possesses all structural features which have been demonstrated to increase antioxidative activity of flavonoids and their *O*-glycosides (Afanasyev et al., 1989).

Wheat, as other cereals, has been known to contain hydroxycinnamic acid derivatives, which demonstrated antioxidative activities (Andreasen et al., 2001). Ferulic acid was reported to be the predominant phenolics acid accounting for approximately 57–77% of total phenolic acids in wheat (Zhou et al., 2004). This acid possesses lower antioxidative capacity than rutin, according to the structural characteristics of these components (Cook and Samman, 1996). This fact could explain the higher AOA of ethanolic extract of buckwheat flours in comparison to the extracts of wheat flours. In addition, Liyana and Shahidi (2007) found that wheat flour possessed the lowest amount of ferulic acid among the different milling fractions of wheat, so this was reflected in its relatively low antioxidative activity.

Ethanolic extracts of buckwheat and wheat flours showed significant ($P < 0.05$) difference in their ability to reduce concentration of DPPH radicals by donating H-atoms from the OH-groups of polyphenols (Brand-Williams et al., 1995), which was confirmed by their IC_{50} values (Figure 3).

DPPH $\cdot$ activities were higher in buckwheat than in wheat flours, indicated by lower IC_{50} values, as the consequence of higher polyphenolics content in

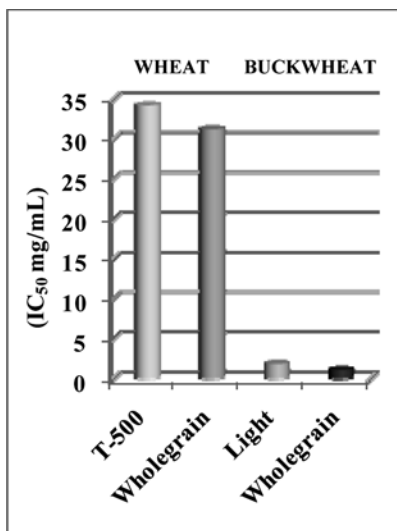


Fig. 3 — Scavenging activity on DPPH $\cdot$

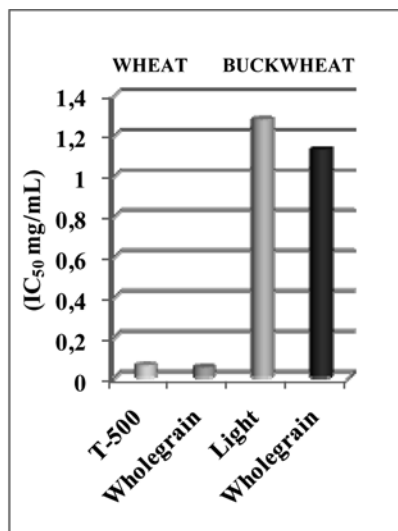


Fig. 4 — Chelating activity on Fe^{2+}

buckwheat flours. Those values were 34.24 and 31.26 mg/mL, and 1.87 and 1.49 mg/mL for wheat and buckwheat flours, respectively.

Reduction of DPPH radicals reveals that examined extracts possess radical inhibitors or scavengers with possibility to act as primary antioxidants. They might react with free radicals, particularly with the peroxy radicals, which are the major propagators of the auto-oxidation chain of fat, thereby terminating the chain reaction (Shahidi and Wanasundara, 1992). Based on the results obtained, the antioxidative activity of investigated extracts, particularly buckwheat extracts, could in part be markedly caused by their radical scavenging properties. Watanabe (1997) and Watanabe and co-workers (1998) reported that flavonoids, first of all rutin, isolated from buckwheat hulls showed radical scavenging activity. Also, Sun and Ho (2005) reported powerful antiradical activity on DPPH radicals of ethanolic extract of whole buckwheat grain, but our results are not completely comparable because of differences in applied methods.

The chelating activity on Fe^{2+} were significantly ($P < 0.05$) higher for wheat flours extracts than for buckwheat flours extracts (Figure 4).

Since ferrous and cupric ions are the most effective pro-oxidant in food systems (Yamaguchi et al., 1988), and ferrous ions are commonly found in food systems, high chelating activity of investigated extracts would be beneficial in retarding metal-catalyzed oxidation (Kehrer, 2000). The possibility of complexing metal ions, as the antioxidant properties themselves, depends on structure of compound, that is in the case of polyphenols (predominant flavonoids from buckwheat and phenolic acids from wheat) implies the number and position of OH- and CH_3O -groups.

Although rutin possesses more structural features than ferulic acid for complexing metal ions, the ethanolic extracts of wheat flours exhibited more potent chelating activity on Fe^{2+} than the buckwheat extracts. Lower antioxidative capacity of rutin as metal chelator in comparison to ferulic acid (and other hydroxycinnamic acids from wheat flour) might be due to steric hindrance of the sugar moiety of rutin. The presence of some more potent chelating component(s) in wheat flour extract might be responsible for its higher chelating capacity. Significant Fe^{2+} chelating activities and inhibitory effects were detected in wheat grain extracts (Yu et al., 2002) which is in correlation with our results presented in the Figure 4. Furthermore, in the Fe^{2+} chelating assay, wheat bran demonstrated superior chelating properties over the other milling fractions, while wheat flour had the lowest chelating capacity (Liyanana and Shahidi, 2007).

CONCLUSION

The obtained results clearly indicated that ethanolic extracts of buckwheat flours (light and wholegrain) possessed good antioxidant properties, including reducing power, scavenging abilities on DPPH radicals and chelating abilities on Fe^{2+} ions. Those extracts showed better antioxidative properties than the ethanolic extracts of wheat flours (type 500 and wholegrain) except when the

chelating activity on Fe^{2+} ions was taken into consideration. Also, wholegrain flours, both wheat and buckwheat, exhibited better antioxidant properties in comparison with light ones.

Concerning all the results, and previously emphasized, buckwheat flours with their antioxidant characteristic as quality criteria, could be used in wheat-based food products and would contribute to their added-value for functional and tailor-made-food production. Besides, buckwheat extract could be used in food as an additive, i.e as a source of natural antioxidants in order to replace the synthetic ones.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science and Technology, Republic of Serbia (Project: TR — 20068).

REFERENCES

- Afanas'ev, I. B., Dorozhko, A. I., Brodskii, A. V., Koostyuk, V. A., Potapovitch, A. I. (1989): *Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation*. Biochemical Pharmacology, 38, 1763—1769.
- Al-Saikhan, M. S., Howard, L. R., Miller, J. C. Jr. (1995): *Antioxidant activity and total phenolics in different genotypes of potato (Solanum tuberosum L.)*. Journal of Food Science, 60, 341—343, 347.
- Andreasen, M. F., Landbo, A-K., Christensen, L. P., Hansen, A., Meyer, A. S. (2001): *Antioxidant effects of phenolic rye (Secale cereale L.) extracts monomeric hydroxycinnamates, and ferulic acid dehydrodimers on human low-density lipoproteins*. Journal of Agricultural and Food Chemistry, 49, 8, 4090—4096.
- Brand-Williams, W. E., Cuvelier, M. E., Berset, C. (1995): *Use of a free radical method to evaluate antioxidative activity*. LWT — Food Science and Technology, 28, 25—30.
- Cody, V., Middleton, E., Harborne, J. B. (eds.) (1986): *Plant Flavonoids in Biology and Medicine, Biochemical, Pharmacological and Structure-Activity Relationships*. New York: Alan R. Liss.
- Cook, N. C., Samman, S. (1996): *Flavonoids — chemistry, metabolism, cardio-protective effects and dietary sources*. Journal of Nutritional Biochemistry, 7, 2, 66—76.
- Decker, E. A., Welch, B. (1990): *Role of ferritin as a lipid oxidation catalyst in muscle food*. Journal of Agricultural and Food Chemistry, 38, 3, 674—677.
- Dietrych-Szostak, D., Oleszek, W. (1999): *Effect of processing on the flavonoid content in buckwheat (Fagopyrum esculentum Möench) grain*. Journal of Agricultural and Food Chemistry, 47, 10, 4383—4387.
- Evans, W. C. (1996): *Trease and Evans' Pharmacology*. London: WB Saunders.

- Holasova, M., Fiedlerova, V., Smrcinova, H., Orsak, M., Lachman, J., Vavreinova, S. (2002). *Buckwheat — the source of antioxidant activity in functional foods*. Food Research International, 35, 2—3, 207—211.
- Hatano, T., Kagawa, H., Yasuhara, T., Okuda, T. (1988): *Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects*. Chemical and Pharmaceutical Bulletin, 36, 2090—2097.
- Hung, P. V., Morita, N. (2008): *Distribution of phenolic compounds in the graded flours milled from whole buckwheat grains and their antioxidant capacity*. Food Chemistry, 109, 325—331.
- Ihme, N., Kieseewetter, H., Jung, F., Hoffmann, K. H., Birk, A., Müller, A., Grützner, K. I. (1996): *Leg oedema protection from buckwheat herb tea in patients with chronic venous insufficiency: a single centre, randomised, double-blind, placebo-controlled clinical trial*. European Journal of Clinical Pharmacology, 50, 443—447.
- Jiang, P., Burczynski, F., Campbell, C., Pierce, G., Austria, J. A., Briggs, C. J. (2006): *Rutin and flavonoid contents in three buckwheat species Fagopyrum esculentum, F. tartaricum, and F. homotropicum and their protective effects against lipid peroxidation*. Food Research International, 40, 3, 356—364, 2007.
- Kehrer, J. P. (2000): *The Haber-Weiss reaction and mechanisms of toxicity*. Toxicology, 149, 43—50.
- Li, S. Q., Zhang, Q. H. (2001): *Advances in the development of functional foods from buckwheat*. Critical Reviews in Food Science and Nutrition, 41, 6, 451—464.
- Liyana-Pathirana, C. M., Shahidi, F. (2007): *Antioxidant and free radical scavenging activities of whole wheat and milling fractions*. Food Chemistry, 101, 1151—1157.
- Oomah, B. D., Mazza, G. (1996): *Flavonoids and antioxidative activities in buckwheat*. Journal of Agricultural and Food Chemistry, 44, 7, 1746—1750.
- Oyaizu, M. (1986): *Studies on products of browning reaction — Antioxidant activities of products of browning reaction prepared from glucoamine*, Japanese Journal of Nutrition, Vol. 44, pp. 307—315.
- Shahidi, F., Wanasundara, P. K. J. (1992): *Phenolics antioxidants*. Critical Reviews in Food Science and Nutrition, 32, 67—103.
- Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M. (1999): *Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent*. Methods Enzymology, 299, 152—178.
- Sun, T., Ho, C.-T. (2005): *Antioxidant activities of buckwheat extracts*. Food Chemistry, 90, 743—749.
- Watanabe, M. (1998): *Catechins as antioxidants from buckwheat (Fagopyrum esculentum Möench) groats*. Journal of Agricultural and Food Chemistry, 46, 3, 839—845.
- Watanabe, M., Ohshita, Y., Tsushida, T. (1997): *Antioxidant compounds from buckwheat (Fagopyrum esculentum Moench) hulls*. Journal of Agricultural and Food Chemistry, 45, 4, 1039—1044.
- Wojcicki, J., Samochowiec, L., Gonet, B., Juzwiak, S., Dabrowska-Zamojcin, E., Katdonska, M., Tustanowski, S. (1995): *Effect*

- of buckwheat extracts on free radical generation in rabbits administrated high-fat diet.* Phytotherapy Research, 9, 323—326.
- Yamaguchi, R., Tatsumi, M. A., Kato, K., Yoshimitsu, U. (1988): *Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions.* Agricultural and Biological Chemistry, 52, 849—850.
- Yu, L., Haley, S., Perret, J., Harris, M. (2002): *Antioxidant properties of hard winter wheat extracts.* Food Chemistry, 78, 457—462.
- Zhou, K., Yu, L. (2004): *Effect of extraction solvent on wheat bran antioxidant activity estimation.* LWT — Food Science and Technology, 37, 717—721.
- Zielinski, H., Kozłowska, H. (2000): *Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions.* Journal of Agricultural and Food Chemistry, 48, 6, 2008—2016.

АНТИОКСИДАТИВНА АКТИВНОСТ БРАШНА ОД ПШЕНИЦЕ И ХЕЉДЕ

Ивана Ј. Седеј, Маријана Б. Сакач, Александра Ч. Мишан,
Анамарија И. Мандић

Институт за прехранбене технологије Нови Сад, Универзитет у Новом Саду,
Булевар цара Лазара, 1, 21000 Нови Сад, Србија

Резиме

Антиоксидативна активност пшеничног брашна (тип 500 и интегрално) и хељдиног брашна (бело и интегрално) одређена је применом 1,1-дифенил-2-пикрилхидразил (DPPH·) теста, на основу мерења редукционе активности и хелатационе активности на Fe^{2+} . Такође, одређен је и садржај укупних фенола у испитиваним етанолним екстрактима.

Садржај полифенола (изражен као еквивалент галне киселине, GAE) у пшеничним брашнима је износио 37,1 и 137,2 $\mu\text{g GAE/g}$ екстракта, док је садржај у брашнима од хељде био најмање четири пута виши и варирао од 476,3 и 618.9 $\mu\text{g GAE/g}$ екстракта. Етанолни екстракти брашна од хељде су показали вишу антиоксидативну активност у свим тестовима, осим за хелатациону активност.

На основу свих добијених резултата може се закључити да обогаћење пекарских производа хељдиним брашном може допринети функционалности производа.

*Rudolf R. Kastori¹, Ivana V. Maksimović¹,
Radovan Z. Marinković², Tijana M. Zeremski-Škorić²,
Jordana N. Ninkov², Marina I. Putnik-Delić¹*

¹ Faculty of Agriculture Novi Sad, Trg D. Obradovića 8, 21000 Novi Sad, Serbia

² Institute of Field and Vegetable Crops, M. Gorkog 30, 21000 Novi Sad, Serbia

GENETIC VARIABILITY OF CONCENTRATION OF MICROELEMENTS IN WILD SUNFLOWER SPECIES AND HYBRIDS*

ABSTRACT: The aim of this work was to investigate genetic specificity of sunflower nutrition with microelements. Therefore, concentrations of essential (Zn, B, Mn, Cu, Fe and Ni) and non-essential (Cr, Al, Cd, As, Pb and Ba) micronutrients were analyzed. Five sunflower hybrids the most grown in Serbia and different populations of wild sunflower species originating from North America: *Helianthus neglectus* Heiser (3), *Helianthus agrophyllus* T&G (3), *Helianthus petiolaris* Nutt. (2), *Helianthus annuus* L. (4) were included in the experiment.

Populations of wild sunflower species and hybrids differed significantly with respect to the concentration of analyzed elements. Manganese concentration was significantly higher in hybrids than in wild species. In all genotypes Fe, B and Mn had the highest concentration. Coefficient of variation of microelement concentration depended on genotype and particular element. In wild populations, for essential microelements, it was between 3.7 and 59.5, whereas in hybrids it varied from 10.0 to 48.8.

Coefficient of variation of concentration of non-essential microelements in wild populations varied from 7.7 to 73.8, and in hybrids from 15.1 to 48.8.

Average coefficient of variation in both wild species and hybrids was the lowest for Mn and Pb. It was the highest for Cr, Ni, and Zn in hybrids and for Cd, Ni, and Cr in wild species.

The results suggest that genetic specificity with respect to uptake of microelements in wild species and hybrids is highly expressed. Broad genetic variability of concentrations of microelements in wild species and hybrids indicate that their reactions to deficiency and/or excess of those elements probably are not the same either. This finding may be used in breeding process aimed specifically at improvement of tolerance and capacity to accumulate microelements in sunflower. Phytoremediation technology designed to reduce the amount of microelements in the soil could thus be advanced by utilization of such plants.

KEY WORDS: wild sunflower species, populations, hybrids, essential, non-essential, microelement concentration

* The paper presents a part of the results obtained during the researching conducted under the project *The study of the genetic specificity of mineral nutrition of hybrids and wild sunflower species* financed by the Ministry of science and Technological Development of the Republic of Serbia.

INTRODUCTION

Plants belonging to the genus *Helianthus* are widely present all over our planet. Various climatic and edaphic conditions resulted in appearance of species that are variable in their morphology and biological characteristics, one of which is mineral nutrition. Breeding process imposed by human activities stimulated an increase in genetic variability of mineral nutrition of some species and genotypes of sunflower. The first reports about varietal differences in plant nutrition were published in the middle of last century (Vose, 1963). Since then, in many studies the existence of genetic variability in mineral nutrition between species and genotypes was confirmed (Epstein, 1972; Klimoševski, 1974; Sarić, 1981). This specificity refers to the temporal dynamics of nutrient uptake and accumulation, plant sensitivity to nutrient shortage or excess, nutrient distribution in plants, etc. (Kastori, 1983).

In sunflower, genetic specificity of mineral nutrition was examined prevalently in different lines and hybrids (Foy et al., 1974; Sfredo et al., 1985; Kastori and Stanković, 1985; Vrebalov, 1987; Krstić and Sarić, 1991; Kastori et al., 2008), and to a much lesser extent in wild sunflower species (Krstić and Sarić, 1987; Seiler and Campbell, 2004; Seiler and Campbell, 2006). The knowledge of specific nutrient requirements of a crop is important both theoretically and practically. It allows farmers to optimize plant mineral nutrition, which in turn makes room for maximum utilization of genetic potential for yield and quality of various crops and particular genotypes.

Environmental pollution with heavy metals and radionuclides, which results from human activities, has serious implications on the production of safe agricultural products. It was found that sunflower could be successfully employed for decontamination of soils polluted with heavy metals and radionuclides (Adler, 1996). In Ukraine, 30 km from Chernobyl, rhizofiltration was used together with phytoextraction for decontamination, and the best result was obtained with sunflower (Sorochinsky, 1998). Dushenkov et al. (1995) found in the laboratory that within 24 h roots of sunflower plants were able to substantially reduce the levels of Cd, Cr (VI), Cu, Mn, Ni, Pb, Sr, U (VI), and Zn in water, bringing metal content close to or below the discharge limits. Sunflower is also able to accumulate ^{137}Cs and ^{90}Sr . Roots of sunflower accumulated up to eight times more ^{137}Cs than the roots of timothy (*Phleum pratense* L.) or foxtail (*Alopecurus pratense* L.). The ability of sunflower to accumulate uranium (U) was reported by Salt et al. (1998) and Jovanović et al. (2001). Apart from the fact that sunflower intensely takes up some heavy metals and radionuclides, it also has high biomass, enabling it to accumulate and extract significant amounts of pollutants from the rhizosphere.

Cited results suggest that sunflower may be suitable for remediation of soils and waters polluted with heavy metals and radionuclides. Therefore, the aim of this study was to assess the capacity of some populations of wild sunflower species and hybrids to take up microelements important from agromonomical point of view, i.e. B, Zn, Mn, Cu, Fe, and Ni, but also of the other, potentially toxic elements: Pb, Cd, As, Cr, Al, and Ba.

MATERIAL AND METHODS

Plants were grown on weakly calcareous chernozem of good physical and chemical properties (Tab. 1). At flowering, completely developed upper leaves, which are physiologically the most active (Ćupina and Sakač, 1989), were taken for the analyses. Micronutrient concentration was analyzed in different populations of wild sunflower species from Northern America (number of populations per species is given in parenthesis): *Helianthus neglectus* Heiser (3), *Helianthus petiolaris* Nutt. (2), *Helianthus agrophyllus* T&G (3), *Helianthus annuus* L. (4) as well as in five sunflower hybrids the most grown in Serbia. Micronutrient content was determined by ICP. The data were statistically processed by analysis of variance, calculation of the least significant difference (LSD), standard deviation, and coefficient of variation using Statistica 8 computer program.

Table 1. Basic soil properties

Depth (cm)	pH		CaCO ₃ %	Humus %	Total N (%)	AL-P ₂ O ₅	AL-K ₂ O
	In KCl	In H ₂ O				mg/100 g	
0—30	7.19	8.22	2.76	1.96	0.154	15.0	24.1
30—60	7.37	8.26	4.56	1.95	0.142	6.6	20

RESULTS AND DISCUSSION

Concentration of analyzed essential microelements varied between populations of wild sunflower species as well as hybrids, suggesting that there is significant genetic specificity with respect to uptake and accumulation of microelements in analyzed genotypes (Tab. 2). Average values of microelement concentrations in wild species and hybrids were the most variable with respect to Mn concentration, which was significantly higher in hybrids. Comparison between the results obtained in hybrids with corresponding values for microelements available in the literature, significant discrepancies are noticed. Concentrations of Fe, B and Mn were significantly higher in hybrids while Zn concentration was lower that was found by Robinson (1970, 1973), Pais (1980), and Bergman (1986). Concentrations of essential microelements shown here correspond to the results that in similar agroecological conditions obtained Kovačević (1986). Higher concentration of essential microelements in hybrids suggests that the soil on which plants were grown was, in spite of weak alkaline reaction, well supplied with microelements in the forms available to plants (Ubavić et al., 1993). Especially high was B concentration, which in hybrids was 165 mg/kg DW in average. Sunflower indeed has high requirements for B (Blamey et al., 1978), which is in accordance with our results. In hybrids, the highest variation from the average was found in hybrid NS-H-45, in which concentration of nearly all analyzed elements was significantly lower as compared to the other hybrids. To the best of our knowledge, concentration of microelements in different populations of wild

sunflower species has not been studied thus far. In some populations of *Helianthus agrophyllus* high Fe concentrations were recorded.

Concentration of a microelement in plant tissues signifies the need of a plant for such an element and therefore such data are of primary biotechnical significance. The knowledge about the accumulation of non-essential microelements may help to choose the species and genotypes for phytoremediation of soils polluted with heavy metals. In Tab. 3 are shown concentrations of non-essential microelements in wild sunflower species and hybrids. In all populations of wild species and in hybrids Al had higher concentration than Ba, Cr, Pb, and As whereas concentration of Cd was the least. There was especially high concentration of Al, Pb, and As in some populations of *Helianthus agrophyllus*. Of tested hybrids, the highest concentration of all analyzed elements was found in NS-H-111, and especially high were concentrations of Cr and Al. Relatively high As concentration in analyzed genotypes may be explained by high As concentration in underground waters of South Bačka, where the plants were grown. Accumulation of Pb in plants was most probably enhanced by the vicinity of highway. Concentration of non-essential microelements in sunflower was studied extensively in the past. Simon (1998) and Simon et al. (1999) studied the accumulation and distribution of Cd in sunflower. In leaves of sunflower, grown on calcareous Chernozem, they found the following concentrations of non-essential microelements (mg/kg): As 0.03, Ba 5.4, Cd 0.2, Cr 0.1, and Pb 0.0. These values were much lower than values found in hybrids tested in our experiment. Kádár and Pálvölgyi (2003) found that sunflower accumulated low amounts of tested elements even when high doses were applied to the soil (810 kg/ha). They concluded that, in spite of high biomass production, sunflower is not suitable for phytoremediation of heavy metal-contaminated soils. At the same time, it is necessary to note that cited authors included only one genotype in their study. The data on the concentration of non-essential microelements in wild sunflower species are not known so far.

Results presented here suggest that there is high genetic variability between populations of wild sunflower species and hybrids in the uptake and tissue concentration of essential and non-essential microelements. This is supported by high coefficient of variation of concentration of some microelements in wild species and in hybrids. Coefficient of variation between populations of some wild species was element-dependent and for essential elements varied between 3.7 and 59.5. For hybrids it varied from 10.0 to 48.8. Coefficient of variation depended both on genotype and on the element. Average coefficient of variation between populations of wild species had the following order: Cd>Ni>Cr>As>Al>B>Fe>Zn>Cu>Ba>Pb>Mn, and when hybrids were compared: Cr>Ni>Zn>Al>Fe>As>Cu>B>Ba>Cd>Pb>Mn (Tab. 2, Tab. 3). Although analyzed populations of wild sunflower species are quite distant as compared to hybrids, they all have low coefficient of variation for Mn and Pb concentration, and high for Ni and Cr. Based on presented data, one can speculate that analyzed genotypes also differ in their tolerance to the deficiency and excess of microelements. This feature of wild species can be used in breeding process. However, genotypes in which concentration of microelement(s) was above

the average may be suitable for phytoremediation, especially hybrids, because they produce more biomass.

Tab. 2 — Content of essential microelements in populations of wild sunflower species and hybrids (mg/kg DM)

Genotypes		Zn	B	Mn	Cu	Fe	Ni
Species	Population						
<i>Helianthus agrophylus</i>	1575	27.56	109.0	74.70	14.26	978.2	1.31
	1677	16.27	138.8	78.50	12.13	443.7	2.36
	1317	22.58	165.7	81.75	17.49	852.8	3.52
	Average	22.13	137.8	78.31	14.62	751.6	2.93
	SD	4.94	24.7	2.93	2.44	239.7	1.78
	CV	22.30	17.9	3.75	16.69	31.9	43.85
<i>Helianthus petiolaris</i>	722	20.82	223.1	68.30	8.88	515.0	1.35
	2167	22.58	111.3	59.60	8.10	452.3	0.40
	Average	21.70	167.2	63.95	8.49	483.6	0.87
	SD	1.32	61.3	4.79	0.77	34.5	0.52
	CV	6.11	36.6	7.50	9.04	7.13	59.51
<i>Helianthus neglectus</i>	457	24.63	164.5	77.80	12.79	515.0	1.50
	1363	25.53	91.5	87.55	12.65	426.3	1.20
	1183	17.53	133.9	73.45	8.16	589.4	3.33
	Average	22.56	131.0	79.50	11.20	510.3	2.01
	SD	3.83	30.5	6.85	2.37	73.2	0.98
	CV	16.97	23.3	8.60	21.12	14.3	48.31
<i>Helianthus annuus</i>	2144	15.19	100.8	126.20	6.60	379.8	1.10
	2156	33.15	90.0	82.80	10.72	413.9	1.61
	2038	20.75	144.3	60.75	9.08	415.2	1.13
	2162	22.03	102.4	108.25	8.13	654.7	2.28
	Average	22.78	109.4	94.50	8.63	465.9	1.53
	SD	6.84	21.9	26.01	1.61	115.7	0.51
	CV	30.04	9.9	27.53	18.61	24.8	33.60
LSD	0.05	1.30	5.32	3.23	1.12	22.5	0.35
	0.01	1.74	7.14	4.34	1.50	36.9	0.47
Hybrids							
NS-H-45		8.28	135.0	129.55	9.55	284.6	1.27
NS-H-111		20.99	153.2	152.50	12.72	706.2	3.52
NS-H-Bačvanin		27.55	159.2	127.55	13.98	377.3	1.70
NS-H-Krajišnik		16.42	163.6	162.30	9.59	385.9	1.00
NS-H-Velja		14.06	218.3	135.95	7.10	397.1	1.87
Average		17.46	165.3	141.57	10.59	430.2	1.87
SD		6.74	29.3	14.15	2.56	150.1	0.91
CV		38.61	17.7	10.00	24.21	34.9	48.80
LSD	0.05	1.05	9.63	3.59	0.64	44.2	0.09
	0.01	1.46	13.36	4.98	0.88	61.3	0.13
LSD for species and hybrids							
0.05		1.21	6.44	3.30	0.96	31.1	0.29
0.01		1.61	8.58	4.41	1.28	41.4	0.39

Tab. 3 — Content of non-essential microelements in populations of wild sunflower species and hybrids (mg/kg DM)

Genotypes		Cr	Al	Cd	As	Pb	Ba
Species	Population						
<i>Helianthus agrophylus</i>	1575	0.43	135.80	0.43	1.22	2.84	21.50
	1677	0.10	59.25	0.09	1.07	2.27	15.50
	1317	0.13	117.70	0.13	1.50	2.33	19.99
	Average	0.22	104.25	0.22	1.26	2.48	18.99
	SD	0.16	34.72	0.16	0.23	0.36	2.72
	CV	69.06	33.30	73.86	18.54	14.45	14.29
<i>Helianthus petiolaris</i>	722	1.07	48.25	0.32	1.26	1.08	18.70
	2167	1.57	31.35	0.08	0.98	1.09	15.49
	Average	1.32	39.80	0.20	1.12	1.09	17.09
	SD	0.28	9.31	0.13	0.16	0.08	1.78
	CV	20.99	23.39	65.59	14.19	7.70	10.39
<i>Helianthus neglectus</i>	457	1.75	65.65	0.64	1.04	1.47	21.00
	1363	1.55	60.35	0.21	0.44	1.40	14.01
	1183	1.37	79.20	0.17	1.18	1.68	21.00
	Average	1.89	68.40	0.34	0.88	1.52	18.67
	SD	0.37	8.95	0.23	0.35	0.14	3.51
	CV	19.67	13.08	66.00	39.61	9.32	18.78
<i>Helianthus annuus</i>	2144	1.16	48.95	0.27	1.02	1.47	17.03
	2156	3.17	55.70	0.35	1.16	1.17	19.36
	2038	1.87	56.45	0.14	1.06	1.74	12.73
	2162	3.40	92.80	0.11	0.37	2.07	16.77
	Average	2.40	63.47	0.22	0.90	1.61	16.47
	SD	1.00	18.05	0.10	0.33	0.37	2.54
	CV	41.48	28.44	46.91	36.22	23.24	15.42
LSD	0.05	0.26	4.42	0.01	0.16	0.29	0.67
	0.01	0.34	5.93	0.02	0.22	0.39	0.90
Hybrids							
NS-H-45		2.30	46.30	0.28	0.50	1.54	24.52
NS-H-111		6.86	105.05	0.34	0.59	1.89	28.14
NS-H-Bačvanin		3.11	53.25	0.26	0.43	1.35	17.17
NS-H-Krajišnik		2.68	47.35	0.34	0.82	1.56	20.34
NS-H-Velja		2.83	60.05	0.28	0.53	1.97	23.04
Average		3.55	62.40	0.30	0.57	1.60	22.64
SD		1.74	22.63	0.05	0.14	0.25	3.81
CV		48.80	36.24	15.54	25.20	15.17	16.82
LSD	0.05	0.26	2.53	0.05	0.05	0.17	0.50
	0.01	0.37	3.51	0.08	0.08	0.24	0.70
LSD for species and hybrids							
0.05		0.25	3.81	0.05	0.14	0.25	0.60
0.01		0.33	5.09	0.07	0.18	0.34	0.80

REFERENCES

- Adler, T. (1996): *Botanical Cleanup Crews*. Sci. News. 150: 42—43.
- Bergmann, W. (1986): *Ernährungsstörungen bei Kulturpflanzen*. VEB Gustav Fischer Verlag, Jena.
- Blamey, F. P. C. Mould, D., Kathanson, K. (1978): *Relationships between B deficiency symptoms in sunflower and B and Ca/B status of plant tissues*. Arg. J. 70: 376—380.
- Ćupina, T., Sakač, Z. (1989): *Fiziološki aspekti formiranja prinosa suncokreta*. Poljoprivredni fakultet, Institut za ratarstvo i povrtarstvo, Novi Sad.
- Dushenkov, V., Kumar, N. P. B. A., Motto, H., Raskin, I. (1995). *Rhizofiltration; the use of plants to remove heavy metals from aqueous streams*. Environ. Sci. Technol. 29: 1239—1245.
- Epstein, E. (1972): *Physiological genetics of plant nutrition*. In: Epstein, E. (ed.) Mineral Nutrition of Plants, Principles and Perspectives. John Wiles and Sons, New York.
- Foy, E. D., Ovellana, T. G., Schwarz, J. W., Fleminy, A. L. (1974): *Responses of sunflower genotypes to aluminium in acid soil and nutrient solution*. Agron. J. 66: 293—299.
- Jovanović, Lj., Cupać, S., Janić, V., Sarić, M., Marković, M. (2001): *Usvajanje i distribucija urana u gajenim biljkama*. Zbornik XIV simpozijuma Jugoslovenskog društva za biljnu fiziologiju, Goč, 26.
- Kádár, I., Pálvölgyi, L. (2003): *Mikroelem-terhelés hatása a napraforgóra karbonátos csernozsjom talajon*. Agrochimica és Talajtan. 52: 79—92.
- Kastori, R. (1983). *Uloga elemenata u ishrani biljaka*. Matica srpska, Novi Sad.
- Kastori, R. R., Marinković, R. Z., Sekulić, P. Đ., Maksimović, I. V., Pucarević, M. M. (2008): *Genetic specificity of magnesium nutrition in sunflower*. Matica srpska, Proceedings for Natural Sciences 115: 27—34.
- Kastori, R., Stanković, Ž. (1985): *Ekskrecija korena i sposobnost iskorišćavanja gvožđa različitih hibrida suncokreta*. Zbornik Matice srpske za prirodne nauke 69: 79—88.
- Klimashevski, L. E. (1974): *Problema genotipskekoj specifičnosti kornjevog hranjenja rastenja*. In: Sort i udobrenie, ANSSSR, Irkutsk, 11—53.
- Kovačević, M. (1986): *Uticaj vegetacionog prostora i dubljenja na rastenje suncokreta, produktivnost fotosinteze, akumulaciju organske materije, makro- i mikroelemenata*. Doktorska disertacija, Poljoprivredni fakultet, Univerzitet u Novom Sadu.
- Krstić, B., Sarić, M. (1987): *Genotipska raznolikost koncentracije azota, fosfora i kalijuma divljih vrsta i imbred linija suncokreta*. Savremena poljoprivreda 35: 229—235.
- Krstić, B., Sarić, M. R. (1991): *Concentration of N, P, K, and dry mass in sunflower inbreds as dependent upon mineral nutrition*. Helia 14: 9—18.
- Pais, I. (1980): *A mikrotápanyagok szerepe a mezőgazdaságban*. Mezőgazdasági Kiadó, Budapest.
- Robinson, R. G. (1970): *Sunflower date of planting and chemical composition at various growth states*. Agr. J. 62: 770—772.
- Robinson, R. G. (1973): *Elemental composition and response to nitrogen, of sunflower, and corn*, Agr. J. 65: 318—320.

- Salt, D. E., Smith, R. D., Raskin, I. (1998): *Phytoremediation*. Ann. Rev. Plant Mol. Bio. 49: 643—668.
- Sarić, M. (1981): *Genetic specificity in relation to plant mineral nutrition*. J. Plant Nutr. 3: 734—766.
- Seiler, J. G., Campbell, G. L. (2004): *Genetic Variability for Mineral Element Concentrations of Wild Jerusalem Artichoke Forage*. Crop Sci. 44: 289—292.
- Seiler, J. G., Campbell, G. L. (2006): *Genetic Variability for Mineral Concentration in the Forage of Jerusalem Artichoke Cultivars*. Euphytica 150: 281—288.
- Sfredo, G. J., Saruggi, J. R., Campo, R. J. (1985): *Accumulation of makro nutrients by two sunflower (Helianthus annuus L.) cultivars under field conditions*. Proceedings of XI International sunflower conference. Mar del Plata: 189—194.
- Simon, L. (1998): *Cadmium accumulation and distribution in sunflower plant*. J. Plant Nutr. 2: 341—352.
- Simon, L., Várkonyi, S., Győri, Z. (1999): *Kadmium akkumuláció vizsgálata napraforgó (Helianthus annuus L.) növényben*. Agrokémia és Talajtan, 48: 99—108.
- Sorochinsky, B. V. (1998): *Applications of Phytoremediation Technologies in Real Conditions of the Chernobyl Zone*. Proceeding of the Chernobyl Phytoremediation and Biomass Energy Conversion Workshop: 229—234.
- Ubavić, M., Bogdanović, D., Dozet, D. (1993): *Heavy metals in soils of the Vojvodina Province*. 217—222. In: Kastori, R. (ed.) Heavy metals and pesticides in soil — Heavy metals and pesticides in the soil of the Vovodina Province. Faculty of Agriculture, Institute of Field and vegetable Crops, Novi Sad (Sr).
- Vose, P. B. (1963): *Varietal differences in plant nutrition*. Herbage Abstracts 33: 1—13.
- Vrebalov, T. (1987): *Režim ishrane i intenzitet usvajanja makro i mikro elemenata od strane postojećih hibrida i njihovih roditeljskih komponenata suncokreta*. Zbornik referata XXI Seminara agronoma Instituta za ratarstvo i povrtarstvo, Novi Sad, Cavtat: 377—388.

САДРЖАЈ МИКРОЕЛЕМЕНАТА У ПОПУЛАЦИЈАМА ДИВЉИХ ВРСТА СУНЦОКРЕТА И ХИБРИДА

Рудолф Р. Кастори¹, Ивана В. Максимовић¹, Радован З. Маринковић²,
Тијана М. Зеремски-Шкорић², Марина И. Путник-Делић¹

¹ Пољопривредни факултет, Нови Сад, Трг Доситеја Обрадовића 8,
Нови Сад, Србија

² Институт за ратарство и повртарство, Максима Горког 30,
Нови Сад, Србија

Резиме

У циљу проучавања генетске специфичности исхране сунцокрета микроелементима испитан је садржај неопходних (Zn, B, Mn, Cu, Fe и Ni) и не неопходних микроелемената (Cr, Al, Cd, As Pb и Ba) у пет најраспрострањенијих хибрида сунцокрета у Србији као и у различитим популацијама дивљих врста сунцокрета пореклом из Северне Америке: *Helianthus neglectus* Heiser (3), *Helianthus agrophyllus* T&G (3), *Helianthus petiolaris* Nutt. (2), *Helianthus annuus* L. (4).

Испитиване популације дивљих врста сунцокрета и хибрида значајно су се међусобно разликовале у погледу садржаја испитиваних микроелемената. Највећа разлика између популација дивљих врста сунцокрета и хибрида утврђена је у садржају Mn који је код хибрида био значајно већи. Код свих испитиваних генотипова највећи је био садржај Fe, B и Mn. Коефицијент варијације садржаја микроелемената зависио је од генотипа и елемента. Код популација дивљих врста сунцокрета кретао се од 3.7 до 59.5, а код хибрида од 10.0 до 48.8. За неесенцијалне микроелементе код дивљих популација коефицијент је био између 7.7, и 73.8, док је код хибрида варирао од 15.1 до 48.8. Коефицијент варијације био је најмањи код Mn и Pb код дивљих врста и хибрида. Код хибрида највећи коефицијент варијације имали су Cr, Ni и Zn, а код дивљих врста Cd, Ni и Cr.

На основу добијених резултата може се закључити да је генетичка специфичност у погледу усвајања неопходних и других микроелемената код популација дивљих врста сунцокрета и хибрида веома изражена. Широка генетска варијабилност испитиваних генотипова у погледу садржаја појединих микроелемената упућује на претпоставку о различитој реакцији према њиховом недостатку и сувишку, што може да буде од значаја у оплеменљивачком раду, посебно при стварању генотипова подесних за фиторемедијацију земљишта загађених микроелементима.

*Borivoj Đ. Krstić¹, Đuro J. Gvozdenović²,
Nataša P. Nikolić¹*

¹ Faculty of Natural Sciences, Department of Biology and Ecology,
Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

² Institute of Field and Vegetable Crops, Maksima Gorkog 30,
21000 Novi Sad, Serbia

GENETIC VARIABILITY OF MINERAL ELEMENTS CONCENTRATION IN PEPPER VARIETIES (*CAPSICUM ANNUUM* L.)

ABSTRACT: Pepper (*Capsicum annum* L.) is one of the major members of the vegetable kingdom. The differences between the individual varieties in mineral nutrition and the uptake of mineral elements have provoked our interest for the investigations of their genotype specificity. The chemical composition of pepper leaves at flowering and at the end of the growing season was analysed. On the average, the content of total ash was app. 22%, namely 19—20% (soluble) and 2—3% (insoluble) at both stages. The highest accumulation values were obtained with nitrogen, then potassium, calcium and phosphorus, whereas the lowest with sodium. The most remarkable genotypic differences were recorded at the end of the growing season. The results obtained clearly demonstrate the chemical heterogeneity of pepper varieties. This crop may be grown successfully only if specificity of its mineral nutrition is known.

KEY WORDS: pepper, variety, mineral elements, concentration diversity

INTRODUCTION

The individual plant varieties show different resistance against high and low temperatures, plant diseases and pests, different length of the vegetation period and different specificity to the individual ions, i.e. elements of mineral nutrition, simply defined as the genotypic specificity of mineral nutrition (Sarić and Loughman, 1983). Content of mineral elements highly correlates with the genetic specificity of plants. The problem of genetic specificity of the content of elements and plant nutrition is of a great importance from both the theoretical and practical aspects. There are many data elaborating this topic (Klimoševski and Černiševa, 1980; Sarić, 1981; Bugevski et al., 2007) while most investigations have been aimed at the differences between the individual genotypes in the content of mineral elements. Among the

plant species investigated are different wheat cultivars, inbred lines of maize and sunflower, as well as many other plant species (Blamey, 1980; Sarić and Kovačević, 1980). A great number of cultivars show differences in the specific content of mineral elements (Sarić and Krstić, 1983). There are also data presenting the investigations on pepper varieties (Somos, 1984; Merkulov et al., 2000). Like with other plant species, there are differences between the pepper genotypes in production of organic matter (Gvozdenović et al., 1996; Gvozdenović et al., 2000) and the requirements for elements of mineral nutrition (Gvozdenović and Takač, 2004). The pepper is an important vegetable being characterized by different varieties intended for different use, i.e. row pepper, ground dried pepper, hot pepper and so on. Its mineral composition is very important particularly when it is used as row pepper. Pepper contains high content of N, K, Ca, P, Mg, Fe, and other elements. There are data showing that edible parts contain 5—6% mineral substances calculated per dry matter (Ćirić, 1973). For it, the present work has as objective to evaluate the chemical heterogeneity of different pepper varieties and to determine the percentage of the individual elements in leaves, i.e. their accumulation.

MATERIAL AND METHODS

The experiments were set up in the greenhouse at Rimski Šančevi, Institute of Field and Vegetable Crops, Novi Sad. Each pepper variety was planted in three replicates, 20 plants per replicate. Before transplanting, experimental plots were fertilized with 10 g NPK (15:15:15)/m². Plants were dressed with 10 g AN (ammonium nitrate) and 10 g NPK/m² four times during the growing season, hoed four times and treated with insecticides two times. Pepper leaves were sampled at flowering and at the end of growing season. Chemical analyses of soil (Table 1) show that it was weak alkaline soil moderate in humus, poor in N and with P and K exceeding tolerant values. High P and K suggests the omission of P and K fertilizers (Kastori, 2000), imposing the need for greater amounts of N.

Tab. 1 — Chemical composition of soil analyzed at the end of growing season

Parameters	Values	Categories	Limiting values
in KCl	7.37	Weak alkaline	7.21—8.20
pH in H ₂ O	7.80	Weak alkaline	7.31—7.80
CaCO ₃ (%)	3.85	Moderate carbonate	2.01—5.00%
Humus (%)	3.01	Moderate humus	3.01—5.00%
Nitrogen (%)	0.150	I group	< 0.200%
P ₂ O ₅ (mg/100 g soil)	107.60	Exceeding tolerant values	> 50.01 mg/100 g soil
K ₂ O (mg/100 g soil)	61.0	Exceeding tolerant values	> 50.01 mg/100 g soil

The pepper variety Plamena, Anita, Amfora, Novosađanka, Lombardija, Una, Vranjska, Krušnica, L-127, and SM-1 were analyzed. Ten standard plants per replication of each variety were sampled.

Nitrogen content in dry matter was determined by the standard Kjeldahl method (Nelson and Sommers, 1973). Total, soluble and insoluble ash was determined in plant material by incinerating dried and ground samples at 450°C (Sarić et al., 1990). Phosphorus concentration was determined by spectrophotometry from the stock solution by the ammonium-vanado-molybdate method (Gericke and Kurmies, 1952) while the content of K, Ca and Na by flame photometry (Sarić et al., 1990) and expressed per dry matter.

The results obtained were statistically processed by the analysis of variance using MSTAT program. Treatments were compared by the Duncan's test, at the significance level of $\alpha = 0.05$, to obtain LSD values. Means of the analyzed parameters were ranked and designated with letters.

RESULTS AND DISCUSSION

One of the factors affecting the production of plant biomass is the concentration of mineral elements. Productivity, i.e. synthesis of organic matter is an important factor in all the plant species and genotypes within these species, followed by the investments primarily in mineral fertilizers. Concentration of ash macroelements and ash content may vary in different varieties and between different plant organs (Sarić and Loughman, 1983). Investigations of mineral composition based on total (raw) ash, as well as soluble (pure) and insoluble ash portions show that the ash content relies on a pepper genotype. Ermakova and Arasimović (1961) quoted that the ash content in edible part of the pepper fruit may vary between 1.03 and 11.82%.

According to the results presented in Table 2, the highest content of total ash in leaves at flowering was obtained with the variety Novosađanka (24.32%) whereas the lowest with SM-1 (20.08%). Based on the results of Duncan's test, tested varieties may be classified into the 6 statistically significant groups.

The highest soluble ash values were recorded in leaves of the variety Novosađanka (21.72%) and the variety Amfora (21.54%) whereas the lowest in the variety Vranjska (19.67%), Lombardija (19.47%) and Una (19.27%). Based on the results of Duncan's test, the tested varieties may be classified into the 5 groups.

The differences between leaves in content of insoluble ash suggest the classification into the 3 statistically significant groups where the highest values of insoluble ash were obtained with the variety Novosađanka (2.60%) and the variety SM-1 (2.53%) whereas the lowest with the variety L-127 (1.44%).

At the end of the growing season (Table 3), the highest total ash was found in the variety Amfora (24.96%) whereas the lowest in the variety Krušnica (19.64%). Based on the results of the Duncan's test, the tested varieties may be classified into the 8 statistically significant groups.

The highest content of soluble ash was recorded in leaves of the variety Amfora (21.78%) whereas the lowest in the variety SM-1 (13.82%). Based on the results of the Duncan's test, the tested varieties may be classified into the 7 groups.

Tab. 2 — Content of ash and macroelements in leaves of different pepper varieties at flowering

Variety	Total ash	Insoluble ash	Soluble ash	N	P	K	Ca	Na
	% in DM			mg % in DM				
ANITA	23.33 ab	2.21 ab	21.12 ab	3.94 bc	382 bc	3729 b	487 a	150 abc
VRANJSKA	21.95 cd	2.28 ab	19.67 c	3.77 c	327 cde	3709 bc	470 a	117 c
NOVOSAĐANKA	24.32 a	2.60 a	21.72 a	4.11 bc	545 a	3875 ab	471 a	104 c
AMFORA	23.88 ab	2.35 ab	21.54 a	4.06 bc	349 cd	4417 a	479 a	121 bc
UNA	20.93 de	1.66 ab	19.27 c	4.22 abc	278 def	3667 bc	241 d	100 c
PLAMENA	22.58 bc	2.04 ab	20.54 abc	4.03 bc	464 ab	3959 ab	325 bc	150 abc
SM-1	20.08 e	2.53 a	17.55 d	4.58 ab	180 g	3959 ab	276 cd	117 c
KRUŠNICA	21.68 cd	1.89 ab	19.79 bc	4.80 a	247 efg	3375 bcd	356 b	121 c
L-127	21.33 cde	1.44 b	19.89 bc	4.29 abc	484 a	3083 cd	330 bc	200 a
LOMBARDIJA	21.07 de	1.61 ab	19.47 c	4.15 abc	215 fg	2833 d	294 bcd	175 ab
Average	22.11	2.06	20.05	4.19	347	3660	373	135
LSD _{0.05}	1.27	0.91	1.32	0.60	85.90	586.60	67.22	50.26

The differences in the content of insoluble ash of the pepper leaves suggest the classification of the tested varieties into the 6 statistically significant groups, where the highest content of insoluble ash was found in the variety Vranjska (3.69%) whereas the lowest in the variety Una (2.59%).

Based on the number of statistically significant groups, one may conclude that the chemical heterogeneity of pepper plants was greater at the end of the growing season when the differences between varieties were greater and the total ash was higher.

Tab. 3 — Content of ash and macroelements in leaves of different pepper varieties at the end of growing season

Variety	Total ash	Insoluble ash	Soluble ash	N	P	K	Ca	Na
	% in DM			mg % in DM				
ANITA	22.37 cd	2.65 c	19.72 bc	3.93 ab	154 c	3438 b	633 d	83 ab
VRANJSKA	22.30 cde	3.69 a	18.61 d	3.59 g	268 ab	2646 c	834 a	83 ab
NOVOSAĐANKA	22.79 b	3.15 abc	19.64 bc	3.87 bcd	267 ab	3563 b	693 cd	75 ab
AMFORA	24.96 a	3.18 abc	21.78 a	3.79 cde	189 c	4021 b	809 ab	92 a
UNA	22.56 bc	2.59 c	19.97 b	4.03 a	163 c	5021 a	685 cd	75 ab
PLAMENA	21.98 de	2.82 bc	19.16 cd	3.74 ef	194 ab	4688 a	760 abc	88 ab
SM-1	17.29 g	3.47 ab	13.82 f	3.54 g	192 c	2688 c	643 d	67 b
KRUŠNICA	19.64 f	3.16 abc	16.48 e	3.74 def	201 c	3625 b	701 cd	71 ab
L-127	21.94 e	2.74 bc	19.21 cd	3.63 fg	257 b	3980 b	693 cd	92 a
LOMBARDIJA	22.02 de	2.67 c	19.35 bc	3.90 abc	307 a	3688 b	701 bcd	83 ab
Average	21.78	3.01	18.77	3.78	229	3736	715	81
LSD _{0.05}	0.39	0.71	0.61	0.13	45.20	534.80	101.60	20.77

Nitrogen is the most widespread element in nature. In green plants, app. 50% of total nitrogen is found in leaf proteins while of that amount, more than 70% is bound in chloroplasts and only 10—20% or less is free in the form of nitrate or ammonia (S a r i ć et al., 1987). There is a close relationship between the growth of vegetative plant parts and the amount of available nitrogen (S o -

mos, 1984). By analysing the nitrogen content in different parts of pepper plants, Kaufmann and Vorwerk (1971) reported on its highest content in leaves (3.6%) and fruit (3.3%) while lower values were recorded in stem and root (app. 2%). According to Marković (1998), the highest N content was found in leaves and fruit (3.63% in leaf dry matter and 3.33% in fruit). Moreover, the highest nitrogen content was found in young pepper plants, gradually decreasing with senescence. Mecs (1974) in his work on seasoning pepper and the percentage of N during its growing reported on its highest content at flowering. Hot varieties contain more nitrogen than sweet ones. By analyzing the variability of the leaf nitrogen content (Tables 2 and 3) it may be concluded that the average nitrogen content was higher (4.19%) at flowering than at the end of the growing season (3.78%). At the flowering stage, the highest nitrogen content was obtained with the variety Krušnica (4.80%) whereas the lowest with the variety Vranjska (3.77%), (5 statistically significant groups). At the end of the growing season, the highest nitrogen content was recorded in the variety Una whereas the lowest in the variety SM-1 (3.54%), (9 statistically significant groups).

In the pepper crop, content of P (essential element) is considerably lower than that of N. Its greatest accumulation is recorded at full flowering. The highest content of this element was recorded in pepper fruit while somewhat lower in leaves, stem, and root (Mecs, 1974). In pepper, phosphorus content is app. 10 times lower than that of nitrogen (200–300 mg %), decreasing from the beginning to the end of growing season. By comparing hot and sweet peppers, Mecs (1974) found that former contain higher phosphorus than latter. Phosphorus means (Tabs 1 and 2) were higher at flowering (347 mg %) than at the end of the growing season (229 mg %), confirming the data published elsewhere (Gvozdenović and Takáč, 2004). The highest P at flowering was recorded in the variety Novosađanka (545 mg %) whereas the lowest in the variety SM-1 (180 mg %). Differences in content of this macroelement were recorded between all the analyzed varieties except Novosađanka and L-127. At the end of the vegetation season, five varieties (Anita, Amfora, Una, SM-1 and Krušnica) showed no differences in content of this element, three varieties (Vranjska, Novosađanka and Plamena) exhibited somewhat higher P content with no mutual differences between varieties whereas the highest P was recorded in the variety L-127 (257 mg %) and Lombardija (307 mg %).

The role of K in plant respiration, photosynthesis, and synthesis and transport of carbohydrates, being essential for high yield and high quality of vegetables was analyzed (Kastori, 1991). This element is present in plants in the form of ions, loosely bound to the protoplasmic colloids, as well as in the form of organic and inorganic salts (Sarić et al., 1987). The pepper crop contains considerable amounts of potassium. Its requirements for this element range from 140 to 210 kg/ha (Kastori, 1991). According to Somos (1984), the largest portion of potassium is present in leaves (4700–6800 mg %) and stem (2600–4800 mg %) while its content in root is between 1900 and 3200 mg %. Potassium content in fruit is rather low, ranging from 2700 to 3400 mg %. The absolute amount of potassium in fruit showed low variations during the growing season (Kaufmann and Vorwerk, 1971). In our in-

vestigations (Table 2), flowering values of potassium were classified into the 7 statistically significant groups. Potassium content in leaves ranged from 4417 mg % (Amfora) to 2833 mg % (Lombardija). Irrespective of pepper variety, averages amounted 3660 mg %. Average value at the end of the growing season was 3 660 mg %. The highest content was found in the variety Una (5 021 mg %) whereas the lowest in the variety SM-1. No significant variations between varieties were obtained, except the variety Una and SM-1.

The role of Ca in life processes of plants has many forms (Mix and Marschner, 1976). The most important is maintenance of structure and function of cell membranes, nonspecific activation of certain enzymes, acid neutralization, and its impact on swelling of protoplasmic colloids (Kastori, 1983). The highest Ca content is recorded in leaves, then stem and root whereas the lowest in seed. Its concentration increases with senescence (Sarić et al., 1987). During ripening, Ca content decreases in fruit whereas increases in leaves. According to Marković (1998), Ca content relies on pepper variety and plant organ, ranging from 0.60% to 3.60%. Our results (Table 2) show significant differences between the average Ca content in leaves at flowering (373 mg %) and at the end of the growing season (715 mg %).

The content of Na in plants relies on a great number of factors like plant species and environmental conditions. Na controls the osmotic pressure and takes part in neutralization of acids originated from different processes in plants. The uptake of Na ions increases with plant senescence (Sarić et al., 1987). According to Sanchez-Conde (1970), sodium content in pepper plants may range from 1 to 2% compared with dry matter whereas Frederick et al. (1962) quoted app. 10 mg %. The results presented in Table 2 show that the Na concentration in leaves showed far less values when compared with other elements. Its average content at flowering was 81 mg % while at the end of growing season it was 135 mg %. When compared with other macroelements, Na exhibited the smallest variability, irrespective of pepper variety and ontogenetic phase. Due to high requirements for mineral elements and supplementary irrigation, lengthen growing season, and genotype differences, for high yields this crop requires application of app. 8—120 kg/ha of nitrogen, 65—100 kg/ha of phosphorus and 140—210 kg/ha of potassium (Kastori, 1991; Lazić et al., 1998).

CONCLUSION

Differences between pepper varieties in content of ash and mineral elements in leaves at flowering and at the end of growing season were recorded in this study. No significant differences between varieties in the content of insoluble ash, irrespective of developmental stage, were found while the highest total ash was found in leaves of variety Novosadanka and Amfora. As for the content of mineral elements in pepper leaves, nitrogen is ranked first, then potassium, calcium and phosphorus whereas sodium is the last. The results obtained clearly demonstrate the chemical heterogeneity of pepper varieties. This crop may be grown successfully only if specificity of its mineral nutrition is known.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Sciences of the Republic of Serbia, Project TR-20075.

REFERENCES

- Blamey, F. P. C., Vermeulen, W. J., Chapman, J. (1980): *Variation in leaf chemical composition of sunflower cultivars and inbred lines*. Communications in Soil Science and Plant Analysis, 11: 1067—1075.
- Bugarski, D., Gvozdenović, Đ., Červenski, J. (2007): *Macro-elements in pepper fruits*. ISHS Acta Horticulture 729, III Balcan Symposium on Vegetables and Potatoes: 193—198.
- Ćirić, D. (1973): *Prilog poznavanju aromatičnih materija paprike*. Doktorska disertacija, Tehnološki fakultet u Novom Sadu. Novi Sad, p. 255.
- Ермакова, А. И., Арасимович, В. В. (1961): *Биохимия овощных культур*. Изд-во Сельскохозяйственной литературы, Журналов и Плакатов, Ленинград.
- Frederick, D., Howard, A., Mac Gillvray, H. J., Yamaguchi, M. (1962): *Nutrient composition of fresh California grown vegetable*, California Agriculture Experiment Station Bulletin 788: 1—43.
- Gericke, S., Kurmies, B. (1952): *Die Kolorimetrische Phosphorsäurebestimmung mit Ammonium-Vanadat-Molybdat und ihre Anwendung in der Pflanzenanalyse*, Zeitschrift für Pflanzenernährung, Düngung, Bodenkunde, Band 59, Heft 3: 32—35.
- Gvozdenović, Đ., Vasić, M., Bugarski, D., Gvozdenović-Varga, J., Takač, A., Jovičević, D., Červenski, J. (1996): *Stanje i perspektive selekcije povrća*. Zbornik radova, Naučni institut za ratarstvo i povrtarstvo, Novi Sad, 25: 455—466.
- Gvozdenović, Đ., Krstić, B., Nikolić, N., Pajević, S. (2000): *Morfološke karakteristike i prinos sorti paprike*. Zb. rad. PMF ser. biol. 29: 17—24.
- Gvozdenović, Đ., Takač, A. (2004): *Paprika*. Idavačka kuća Draganić, Beograd.
- Kastori, R. (1983): *Uloga elemenata u ishrani biljaka*, Matica srpska, Novi Sad.
- Kastori, R. (1991): *Kalijum je neophodan za visok prinos i dobar kvalitet povrća*. International Potash Institute, Basel, Switzerland.
- Kastori, R. (2000): *Bogatstvo zemljišta kalijumom i efikasnost primene kalijumovih đubriva u Jugoslaviji*. Country Report 14. International Potash Institute, Basel, Switzerland.
- Kaufmann, H. G., Vorwerk, R. (1971): *Zur Nährstoffaufnahme von Gemüsepaprika*. Arch. Gartenbau 19: 7—27.
- Marković, V., Vračar, Lj. (1998): *Proizvodnja i prerada paprike*, Feljton, Novi Sad, p. 202.
- Mécs, J. (1974): *Nutrient uptake in spice paprika*. Zöldségterm. Kut. Int. Bull. 9: 137.

- Merkulov, Lj., Gvozdenović, Đ., Krstić, B., Krstić, L. (2000): *Uporedna anatomska analiza perikarpa nekih sorata paprike (Capsicum annum L.)*. Zb. rad. PMF ser. biol., 29: 48—55.
- Mix, G. P., Marschner, H. (1976): *Einfluss exogener und endogener Faktoren auf den Calciumgehalt von Paprika und Bohnenfrüchten*, Zeitschr. f. Pflanzenernährung, Bodenkunde 139: 551—563.
- Nelson, D. W., Sommers, L. E. (1973): *Determination of total nitrogen in plant material*. Agronomy Journal 65: 109—112.
- Sanchez-Conde, M. P. (1970): *Respuesta de la planta de pimiento ante distintos tratamientos de potasio o de nitrógeno*. Anales de Edafología y Agrobiología 39: 503—516.
- Sarić, M. R., Kovačević, V. (1980): *Genetska specifičnost mineralne ishrane kukuruza*. U: Fiziologija kukuruza (Ed J. Belić), SANU, Beograd, pp. 127—143.
- Sarić, M., Krstić, B. (1983): *Role of genetic specificity of mineral nutrition of plants in increasing economical production of organic matter*. Biotech. 83, London, 4—6 May.
- Sarić, M., Krstić, B., Stanković, Ž. (1987): *Fiziologija biljaka*, Naučna knjiga, Beograd, pp. 264—315.
- Sarić, M. R., Loughman, B. C. (Eds) (1983): *Genetic aspects of plant nutrition*. Martinus Nijhoff/Dr Jung Publishers, The Hague/ Boston/ Lancaster.
- Sarić, M., Petrović, M., Krstić, B., Stanković, Ž., Petrović, N. (1990): *Praktikum iz fiziologije biljaka*, Naučna knjiga, Beograd, p.245.
- Somos, A. (1984): *The Paprika*, Akadémia Kiadó, Budapest, p. 293.

ГЕНЕТИЧКА ВАРИЈАБИЛНОСТ КОНЦЕНТРАЦИЈЕ МИНЕРАЛНИХ ЕЛЕМЕНАТА КОД РАЗЛИЧИТИХ СОРТИ ПАПРИКЕ (CAPSICUM ANNUUM L.)

Боривој Ђ. Крстић¹, Ђуро Ј. Гвозденовић², Наташа П. Николић¹

¹ Природноматематички факултет, Департман за биологију и екологију,
Трг Доситеја Обрадовића 2, 21000 Нови Сад, Србија

² Институт за ратарство и повртарство, Максима Горког 30,
21000 Нови Сад, Србија

Резиме

Паприка (*Capsicum annum L.*) представља једну од водећих повртарских култура а како се поједине сорте међусобно разликују по захтевима за минералном исхраном као и изношењем елемената из земљишта приносом потребно је испитати њихову генотипску специфичност. У раду је специфичност минералне исхране испитана одређивањем хемијског састава листа у фази цветања и на крају вегетације. Добијени резултати показују да је садржај укупног пепела у просеку износио у обе испитиване фазе око 22%, растворног 19—20%, нерастворног 2—3%. На основу резултата може се закључити да се у паприци од свих макроелемената највише акумулира азот, одмах затим следе калијум, калцијум, фосфор, док натријума има процентуално најмање. Генотипске разлике нарочито су изражене на крају вегетације. Резултати указују да је у циљу успешне производње паприке неопходно водити рачуна о специфичним потребама сорти за минералним материјама.

*Rudolf R. Kastori¹, Ivana V. Maksimović¹,
Tijana M. Zeremski-Škorić²,
Marina I. Putnik-Delić¹*

¹ Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

² Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia

RARE EARTH ELEMENTS — YTTRIUM AND HIGHER PLANTS*

ABSTRACT: Rare earth elements (REEs) form a chemically uniform group with very similar physical and chemical properties. The REEs include the elements scandium, yttrium, and the lanthanides from lanthanum to lutetium. They are widely distributed and present in all parts of the biosphere. REEs are required in industry, agriculture, medicine, biotechnology, environmental problems and many other fields. Lately, many experiments show their positive or negative, first of all nonspecific, effect on life processes of higher plants as well as growth and yield of cultivated species, but the physiological mechanisms are still not well understood. It has been determined that yttrium is widely distributed in plants, as well as that certain plant species uptake yttrium at different extent. Its highest accumulation is in the root and the leaf. Although yttrium was discovered more than two centuries ago, its effect on higher plants — their anatomical and morphological built, physiological and biochemical processes etc. — is very little known. One of the basic reasons is that yttrium, as well as other REEs elements, according to current knowledge, is not biogenic for higher plants and — wider — for live organisms. The objective of this paper is to concisely show previous knowledge about yttrium in the plant world.

KEY WORDS: Rare earth elements, yttrium, soil, plant, uptake, content, distribution, functions

INTRODUCTION

There are 94 elements in the nature that are more or less present in all parts of the biosphere, but not all of them are biogenic for live organisms. For higher plants, biogenic mineral macroelements are C, N, P, S, K, Ca, and Mg, microelements are Fe, Mn, Zn, Cu, B, Mo, Cl and Ni, and beneficial elements are Na, Si and Co (Marschner, 1995). Numerous papers report that in par-

* The paper presents a part of the results obtained during the researching conducted under the project *Accumulation, distribution, and physiological effect of yttrium in higher plants* financed by the Ministry of science and Technological Development of the Republic of Serbia.

** Review paper.

ticular conditions the elements that are not essential for higher plants can stimulate their growth and development too. Stimulating effects of low concentrations of titanium (Pais, 1983), lead (Diehl et al., 1983), fluorine (Hitchock et al., 1971, Kastori and Petrović, 1983, 1984), chromium (Krstić et al., 1991) and other elements on the growth and development and physiological and biochemical processes of plants were established. Stimulating effects on plants have also been attributed to rare earth elements (REEs). The REEs form a chemically uniform group and include the lanthanides and actinides. Due to many similarities, lanthanides are often marked with the common symbol Ln. The term lanthanides strictly defined refers to 15 elements following lanthanum to lutetium in the periodic table: lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu). Yttrium (Y) and scandium (Sc) are often included in the list of REEs (Tyler, 2004) and scandium (Sc) (Hu et al., 2004). The element Y, which lies above La, and has a similar +3 ion with a noble gas core, (Ar), has atomic and ionic radii close to those for Tb and Dy. It therefore resembles them closely in its chemistry and is generally found in nature with the lanthanides. The lanthanides plus Y are commonly called the rare earths, although many of them are relatively abundant (Cotton and Wilkinson, 1988). Numerous research of REEs also include yttrium, which makes it necessary to review the REEs effect when studying yttrium's effect on organic production and life processes of plants. Even more so, since numerous investigations by now showed REEs effect on physiological and biochemical processes, growth, development and yield of plants (Hu et al., 2004). Detailed review of REEs in biological systems and their implementation is given in works of Brown et al. (1990) and Horovitz (2000).

Physiological effects of rare earth elements on plants

On average content of REEs on the Earth, crust is close to 0.015%. More than 250 kinds of minerals containing REEs are known, among which the best known are: bastnaesite, monazite, xenotime, loparite, euxenite, and parisite. Content of REEs in the soil depends among other things on characteristics of the basic substratum and descends according to the following sequence: granite > quaternary > basalt > purple sandstone > red sandstone. Besides, content of REEs in the surface layer of soil depends also on pedogenetic processes, weather, content of organic matter and clay minerals etc. According to Bohn et al. (1985), content of REEs in the soil is in the range 30–700 mg/kg. Anthropogenic sources of REEs are phosphoric mineral fertilizers, phosphorus plaster, sewer mud, and atmospheric deposition. Origin, content, and dynamics of REEs in the soil are comprehensively considered in works of Tyler (2004) and Hu et al. (2006).

Physiological aspects of REEs were mainly researched by Chinese researchers and the results were mostly published in Chinese language. Detailed

review of these and other researches in the field were given by Hu et al. (2004). Work of the mentioned author presented the basis of summarized knowledge on uptake and effect of the REEs on physiological processes in plants in this review.

Plants can uptake the REEs through the root as well as the aboveground organs. Intensity of uptake of REEs and single elements of this group depends on numerous factors. There have also been determined synergism and antagonism between certain elements of the REEs during uptake. Their mobility in plants also differs. It is considered that Casparian strip of the root limits the transport of the REEs in the root. Use of EDTA encourages uptake of REEs, since this organic ligand increases desorption of REEs in the soil (Yang et al., 1999). Degree of translocation of REEs from the soil into plants is about 20%. They are mostly accumulated in the root, and less in the stem and reproductive organs. They are found in both intracellular and extracellular parts of a plant and form chelate compounds with numerous components of metabolism — amino-acids, nucleic acids, proteins etc. Uptake of REEs through the leaf is much faster than through the root. This is not characteristic only for the REEs, since the ions of other elements have far more faster uptake through the leaf and are included in the metabolism of a plant than when the uptake goes through the root.

Tyler (2005) states increase of concentration of most of the 58 examined elements, among which also REEs, on dry mass basis during the course of beech leaf senescence. According to Tyler and Olsson (2005), no clear relationship between soil properties or REEs content and leaf REEs concentration was detected in eight forest-floor herbaceous plants.

REEs affect the uptake and metabolism of mineral matter in plants. They stimulate the uptake of certain elements, and decrease the uptake of others. Their influence on the uptake and function of calcium was studied the most (Brown et al., 1990). The REEs have very similar chemical properties and ion radius as calcium. They bond at the same places in organism and have a similar effect as calcium. This mainly refers to La^{3+} . By using La it is possible to alleviate symptoms of calcium deficiency and stimulate growth of plants showing deficiency of this element. Similarly to calcium, REEs influence the stability, permeability and functioning of cell membranes and can inhibit calcium uptake (Rengel, 1994).

Hu et al. (2004) state in their paper results of numerous authors who have determined effect of certain REEs on activity of some enzymes, the content of phytohormones, productivity and intensity of photosynthesis, chlorophyll synthesis, translocation of photosynthesis products, water regime of plants and their resistance to water deficiency, symbiotic fixation of atmospheric nitrogen etc. But also, a favorable effect of REEs on seed germination has been determined by a larger number of authors (Chen Shu-Linang et al., 1987, Bai Bao-Zhang et al., 1988).

Research results about the REEs effect on plant growth are somewhat contradictory. The early findings mostly point to inhibitory effect of these elements on plant growth, first of all lanthanum (Pickard, 1970, Van Stevenick et al., 1976). In most papers published lately it is pointed to a sti-

mulating effect of REEs on growth and organic productivity of plants. They were found to affect favorably the yield of many grown crops such as rice (Hung Zhi-Gang et al., 1983), sugar cane (Ning Ja-Ben et al., 1985), sugarbeet (Xie Hui-Guang and Yu Zheng-He, 1986, Bai Bao-Zhang and Wang Ting-Hui, 1988), soybean (Kastori et al., 1990b), sunflower (Bai Bao-Zhang et al., 1990a).

Yttrium in plants

In 1974 Finnish chemist Johan Gadolin has investigated a mineral discovered by Arrhenius in 1787 in Sweden in the village of Ytterby, and obtained an oxide, which he named ytterbia. The product was a mixture of oxides from which in 1843 Mosander obtained yttrium-oxide, and later in 1928 Wöhler managed to obtain the metal yttrium (Greenwood and Earnshaw, 1999).

In nature yttrium can be found in small quantities in polymetal minerals that contain compounds of lanthanides, calcium, iron, uranium, titanium, zirconium etc. The Earth crust contains about 5.0×10^{-3} weight percent of Y. Sources of Y are ores xenotime, gadolinite, samarskite etc. Apollo missions to the Moon showed that there is a very high content of Y in moon rocks (www.graysonhighschool.org). In chemical compounds Y is found in the form of cation Y^{+3} or is a part of complex anions. Y salts are colourless, they hydrolyze, tend to form double and complex salts. It forms inorganic and chelate compounds and alkyl products (Ripán and Ceteanu, 1972).

Interest and demand for Y have suddenly increased since the production of colour TV screens began, for some of its compounds found use as material that glow under stroke of electrons (Ribár, 2006). Yttrium is also used to create fake games, lasers, radar equipment, camera lenses and so much more. Metal Y and its compounds and isotopes are lately used in different contemporary technologies and instrumentation, as well as medicine and biotechnology (Horowitz, 1995). Research on its impact on physiological and biochemical processes in plants are more recent, they began at the end of the last century and are still rather rare.

Content of Y in the soil depends on litogenic and pedogenic processes, chemical composition of the rocks and minerals, physical and chemical properties of the soil and anthropogenic factors. Ultramafic rocks contain somewhat smaller amounts of Y (0.5 to 5 ppm) than acid rocks and sandstones (28 to 55 ppm) (Kabata-Pendias, 2001). According to Erdaman et al. (1976) content of Y in non-cultivated soil in the Missouri area USA is 23 ppm, while in cultivated soil it is 15 ppm. Gough et al. (1988) has determined in different types of soil in Alaska content of Y of < 4 to 100 ppm (DW). Ichihashi et al. (1992) have reported that content of Y in the soil is in the range, have reported that content of Y in the soil is in the range < 10 to 150 mg/kg. Based on the results of numerous authors it can be concluded that the content of Y in the soil is about 20 ppm. In the soil, yttrium forms complexes with proteins and humus matter (Paulenova et al., 2000). The adsorption process of Y-90 in soil was influenced by the soil type and the horizon's depth.

Hydrogen ions did not have a significant influence on the adsorption of Y-90 (Solecki, 2004).

The man enriches the soil with Y with his activities, first of all by usage of mineral fertilizers. According to Kasinova et al. (1996) content of Y in nitrogen mineral fertilizers is the range 2—4 ppm. As said by Kádár (2000) in superphosphates of different year of make the content of Y was in the range 10.4—53.9 mg/kg, in potassium salt 0.07—1.12 mg/kg, while in the investigated nitrogen fertilizers it was present only in traces. Organic fertilizers also enrich the soil with Y. According to investigations by Yoshino and Goto (taken from Kabata-Pendias, 2001) the content of Y in the dry matter of rice straw was 0.061 ppm, while in the animal manure prepared from the same straw it was much higher, 0.73 ppm.

In nuclear fission reactions various isotopes of Y are produced in rather large amounts. Twenty six Y unstable isotopes have been characterized. Y-90 exists in equilibrium with its parent isotope Sr-90, which is a product of nuclear explosions. Because of this, the study of the uptake and accumulation of Y by plants is assumed important. As a result of the Chernobyl disaster in 1986, measurements of Sr-90 and Y-90 and other radionuclides were measured in Hungary. Levels in plants were 10—15 folds higher, respectively, than in the years 1981—1985 (Cseh and Kiss, 1993). The isotopes of Y are not apt to be as important as other fission products in the hazard evaluation of fission product absorption by plants. The uptake efficiency for Y varies from 2 to 4 orders of magnitude less than Sr, depending on the plant and nutrient environment (Rediske and Selders, 1953). According to Klechkovsky and Tselishcheva (1957) plants absorb more radioactive strontium than yttrium, more yttrium than strontium is transported within plants.

First published papers about uptake of Y by plants originate from the beginning of the second half of the last century. At the time a large number of isotopes of different elements was obtained, of Y as well, which enabled their appliance in experimental work too. Spooner (1949) was one of the first to investigate the uptake of radioactive Y in plants. He established that the red and brown algae react differently in their uptake of Y and concluded that the uptake of Y by algae from sea water to be partly adsorption and partly ionic exchange with Y already associated with a cell. According to Nishita et al. (1961) the uptake of several radioactive fission products from contaminated soils by crop plants was different among plant species and conditions of growth. The relative order of magnitude of uptake of fission products by crop plants through the root system appeared to be: $\text{Sr}^{89-90} > \text{J}^{131} > \text{Ba}^{140} > \text{Cs}^{137} > \text{Ru}^{106} > \text{Y}^{91} > \text{Pm}^{147} > \text{Zr}^{95} - \text{Nb}^{95}$. Distribution of fission products in different part of investigated crop plants were: pea leaves 10.0, stems 1.55, pods 0.80, seeds 0.02, roots 385.0, bean leaves 6, stems 2, fruits 2, lettuce leaves 3, stems 1, radish leaves 5, roots 7, carrots leaves 4, roots 4 D.P.S./g. Accumulation of Y was highest in root and leaves of plants, but comparatively low in seeds, fruits, or edible roots. Jacobson and Overstreet (1948) established that dwarf pea plants accumulate Y in their leaves to only about 0.034 times the concentration in the soil. The mentioned fact points to the assumption that Y is strongly bonded in the soil, which makes its uptake and consequently

translocation to aboveground plant organs limited. This is supported also by the research results of Rediske and Selders (1954) which state that barley on sandy loam soil can accumulate only about 0.006 of the concentration of Y present in the soil (concentration in aerial tissues/concentration in soil). Some authors claim far larger concentration factor for REEs. Citing results of other authors, Hu et al. (2004) state that the natural translocation rates for REEs from soil to plant are approximately 20%, and for REEs fertilizer „Changle” this rate was 55—60%. According to Welch (1984) biological absorption coefficient (plant /soil ratio for the plant concentration of a given element plotted as a function of its concentration in the soil) for Y and other REE elements in terrestrial plants is 0.003. Dobrovolsky (1994) calculated higher value of 0.15.

The uptake and consequently accumulation of Y in plants is affected by numerous biotic and abiotic factors. Presence of chelating agents in calcereous soil encouraged the Y uptake in beans (Essington et al., 1963). The pH value of the environment also affects the Y uptake. According to Tyler and Olsson (2001) the concentration of all REEs, except Y, in the roots of the grass *Agrostis capillaris* was inversely and linearly related to the pH of the soil solution. The result of Rediske and Selders (1954) show that within the range of pH the uptake efficiency was proportional to the hydrogen ion concentration. The same authors established that the uptake into the aboveground parts of the bean plants and the amount of Y associated with the roots was proportional to the concentration of Y added to the nutrient solution. Earlier, Robinson (1943) established that entering gadolinite into the soil increases the uptake of REEs. According to Gu et al. (2000) in solution culture the uptake of Y increased. Ozaki et al. (2002) investigated the uptake mechanisms of Y and REEs for accumulators and non-accumulators species. Autumn fern (*Dryopteris erythrosora*) accumulator species showed no ionic-radius dependence of Y-REE uptake by leaves, while non-accumulator species showed an extremely high uptake for Y compared to REEs. Y-REE uptake by autumn fern was influenced by the addition of chelating reagents to the uptake solution, while no effect was observed for non-accumulators species.

The Y content in certain plant species was investigated by a larger number of authors. Based on the published results, it can be concluded that certain plant species accumulate this element at different extent. The Y content in plants was thoroughly investigated by Connor and Shacklette (1975) and Shacklette et al. (1978). These authors have determined in 10% of the investigated species a measurable concentration of Y. In edible plants the Y content is in the range 20—100 ppm (AW), the highest value was determined in cabbage. Cowgill (1989) investigated 71 plant species that belong to 29 families and established presence of Y in all of the 1100 analyzed samples. This big difference in distribution of Y in plants between results of certain authors most likely lies in different sensitivity of the applied determination methods and genetic specificity of the investigated species regarding Y accumulation. Based on what we know today, it can be said that Y is very abundant in the plant world. Compared to other plant species, ferns and lichens have a high content of Y (Ichihashi et al., 1992). That is confirmed

by Erämetsä and Yliroukanen (1971) who have established that Y concentration in mosses and bryophytes is in the range 2—200 ppm (DW). Far lower values for the same plants are stated by Bowen (1979) — for lichens from 0.2 to 2 ppm and for bryophytes from 1.3 to 7.5 ppm (DW). These results confirm that beside the genetic specificity of the species and probably the genotype, an important role in Y accumulation belongs to ecological factors, which may explain significant differences in Y content determined at the same species in different environments by certain authors. Duke (1970) has stated that the content of Y in food plants from tropical forest region goes from 0.01 to 3.5 ppm (DW). Relatively high concentrations of Y exist in species of the phylum *Mycophytophyta* (Horovitz, 1993). Comparing the stated values of Y content in plants to the values of some significant biogenic microelements (Mo, Co, Se, Ni etc.), it can be concluded that by commonness in plants they are very similar to yttrium.

Distribution of elements in plants depends on their mobility in the plant, physiological and biochemical role, morphological and anatomical properties, growth and development phase etc. According to Zhu and Chen the sequence of some REEs in plants is as follows: $^{147}\text{Nd} > ^{141}\text{Ce} > ^{90}\text{Y} > ^{140}\text{La}$ (taken from Hu et al., 2004). Reiske and Sedlers (1954) point to poor translocation of Y from aerial tissues and translocation from the root. For REEs elements it can be generally said that their distribution in plant organs is as follows: root > leaf > stem > flower > fruit. According to Hong et al. (1999) Y is mostly accumulated in the root, than the leaf and the least in the stem. A significant accumulation of Y in the beans root was detected also by Rediske and Selders (1954). The mentioned authors believe that the large accumulation of Y in the root is not the result of the life activity of the root tissue, but firstly of its adsorption onto the surface of the root system.

Yttrium is not a biogenic element for higher plants, why it is understandable that there is very little data in literature on its affect on growth, development, and life processes of plants. The capacity of Y bonding to biomolecules is indicated by the formation of complexes with phosphorus-containing compounds, mucopolysaccharides and fluoride (Horovitz, 2000) and nucleic acids (Fujiiwara et al., 1990). The first paper about the influence of Y on plant growth was published at the beginning of the century by Evans (1914). This author established that 1.7 mg Y^{3+}/l produced a diminution in cell division with irregular cell arrangements by hyacinth (*Hyacinthus* sp.) rootlets. Cytological studies of the effect of a 40 metal solution, among which 0.5 up to 5 mM of yttrium sulfate on root meristem of *Allium cepa* showed a common effect of colchicine type mitosis (Levan, 1945). According to tests performed by Young (1935) appliance of 500 mg Y^{3+}/kg sandy loam affected stimulating on the growth of grass timothy (*Phleum pratense*). Fänrich (1964) established that yttrium chlorides combined with boron either had no effect or showed a slight inhibitory effect. Appliance of solution of 50 mg/l of the single REEs La, Pr, Nd, Sm, Eu, Gd and Y as RECl_3 on seeds, increased germination of wheat by 1.3 to 16.5% (Jie, taken from Hu et al., 2004).

Numerous data from literature point to a wide abundance of REEs (Hu et al., 2006) as well as to their physiological and biochemical effect on plants

(Hu et al., 2004). They are not biogenic for higher plants or for other live organisms, but can specifically influence their life processes. Experiments, performed mainly in China, show that the REEs can be beneficiary for organic plant production (Hu et al., 2004). Even with numerous results (Horowitz, 2000), it can still be said that it is not clear enough how they affect human and animal health, which makes it necessary to investigate further in this field, having in mind that by being applied in plant production they enter the food chain. There is far less data in the literature about the single affect of certain REEs elements on life processes of higher plants, which goes for Y too (Horowitz, 1993, 1995, 2000, Kabata-Pendias, 2001). Identifying the uptake, accumulation, distribution of REEs in certain plant species and therefore their entrance into the food chain, as well as their non-specific stimulating or toxic effect on plants, can be very significant ecologically too. Therefore, an increased interest of researchers lately for this group of microelements is understandable. What certainly contributed to this trend is the existence of new and much more sensitive determination methods.

REFERENCES

- Bai Bai-Zhang, Kastori, R., Milošević, M. (1988): *Effect of the elements from the scandium group on seed germination and seedling growth in soybean, sunflower, and sugarbeet*. Zemljište i biljka, 37: 207—215 (Sr).
- Bai Bao-Zhang, Wang Ting-Hui (1988): *Effect of rare earth elements on the productivity of sugar beet*. China Sugar Beet J. 1: 20—26.
- Bai Bao-Zhang, Kastori, R., Petrović, N. (1990a): *Effect of elements from scandium and lanthanid groups on growth and morphological chracters of young sunflower plants*. Helia, 13: 1—9.
- Bai Bao-Zhang, Kastori, R., Petrović, N. (1990b): *Effect of elements from scandium and lanthanid groups on some morphogical and physiological characters of young soybean plants Glycine max. (L.) Merrill*. Agrochimica, XXXIV. 467—474.
- Bohn, R. L., McNeal, B. L., O'Conner, G. A. (1985): *Soil Chemistry*, 2nd Edition, John Wiley & Sons, New York.
- Bowen, H. J. M. (1979): *Environmental Chemistry of the Elements*. Academic Press, New York.
- Brown, P. H., Rathjen, A. H., Graham, R. D., Tribe, D. E. (1990): *Rare earh elements in biological systems*. In: Gschneider, K. A., Eyring, L. (eds.) Handbook on the Physics and Chemistry of Rare Earths. Elsevier Sciences Publisher, New York, Vol 13, 423—453.
- Chen Shu-Liang, Tang Shu-Yan, Lio Li-Xia (1987): *Research of yielding and physiological effects of rare earth elements on soybean*. Jilin Agri. Sci. 4: 41—47.
- Connor, J. J., Shacklette, H. T. (1975): *Background geochemistry of some rocks, soils, plants and vegetables in the conterminous United States*. U.S. Geol. Surv. Prof. Pap. 547f, 168.

- Cotton, A. F., Wilkinson, G. (1988): *Advances in Organic Chemistry*. John Wiley & Sons, New York.
- Cowgill, U. M. (1989): *The chemical and mineralogical content on the plants of the Lake Huleh Preserve*. Israel Phil. Trans. Roy. Soc. London, 326B: 59—118.
- Cseh, E., Kiss, B. (1993): *Observations of the amount of Sr isotopes and their changes in different samples after Chernobil disaster*. Élelmisz. Közl. 37:226—231.
- Diehl, K. H., Rosopulo, A., Kreuzer, W., Judel, G. K. (1983): *Das Verhalten von Bleitetraalkylen im Boden und deren Aufnahme durch Pflanzen*. Z. Pflanzener. Bodenk. 146: 551—562.
- Dobrovolsky, V. V. (1994): *Biogeochemistry of the World's Land*. Mir Publishers, Moscow, 36—40.
- Duke, J. A. (1970): *Ethnobotanical observations on the Chocó Indians*. Econ. Bot. 23: 344—351.
- Element of the year # 3: Yttrium*: www.graysonhighschool.org
- Erämaetsä, O., Yliroukanen, L. (1971): *The rare earths in lichens and mosses*. Suom. Kemistil., 44: 121—127.
- Erdman, J. A., Shacklette, H. T., Keith, J. R. (1976). *Elemental composition of selected native plants and associated soils from major vegetation-types areas in Missouri*, US. Geol. Survey Prof. Pap, 954c, 30.
- Essington, E., Nishita, H., Wallace, A. (1963): *Effect of the chelating agents on the uptake of Y-91, Ru-106, Ce-144 and Pm-147 by beans grown in a calcareous soil*. Soil Sci., 95: 331—337.
- Evans, W. H. (1913): *The influence of the carbonates of the rare earths (Cerium, Lanthanum, Yttrium) on growth and cell-division in hyacinths*. Biochem. J., 7: 349—355.
- Fährnich, P. (1964): *Investigations on the influence of boron on pollen germination and on growth of pollen micella*. In: Linskens, H.F. ed. *Pollen Physiology and Fertilization*. North-Holland, Amsterdam, 120—127.
- Fujiwara, K., Kojyo, R., Okada, K. (1990): *Coprecipitation of trace metals by DNA and RNA molecules*. Anal Chem., 62: 504—508.
- Gough, L. P., Secerson, R. C., Shacklette, H. T. (1988): *Element concentration in soil and other surficial materials of Alaska*, US Geol. Survey Prof. Pap. 1458, 53.
- Greenwood, N. N., Earnshaw, A. (1999): *Az elemek kémiája*. Nemzeti Tankönyvkiadó, Budapest.
- Gu, Z., Wang, X. R., Cheng, J., Wang, L. S., Dai, L. M. (2000): *Effects of sulfate on speciation and bioavailability of rare elements in nutrition solution*. Chem. Spec Bioavailab. 12: 53—58.
- Hitchcock, A. E., McGune, D. C., Weinstein, L. H., Maclean, D. D., Jacobson, J. S., Mandl, R. H. (1971): *Effects of hydrogen fluoride fumigation on alfalfa and orchard grass: A summary for experiments from 1952 through 1965*. Contrib. Thompson Inst. 24: 363—372.
- Hong, F. S., Wei, Z. G., Tao, Y., Wang, S. K., Yang, Y. T., Cao, X. D., Zhao, G. W. (1999): *Distribution of rare elements and structure characterization of chlorophyll-lanthanum in a natural plant fern*. Acta Bot. Sinica, 41: 851—854.

- Horovitz, Ct. T. (1993): *Could scandium and yttrium be required for life?* In: Anke M., Meissner, D., Mills C. F. (eds.) *Trace Elements in Man and Animals*. TEMA-8, 747—749.
- Horovitz, T. C. (1995): *Two hundred years of research and development of yttrium*. *Trace elements and Electrolytes*, 12: 153—160.
- Horovitz, T. C. (2000): *Biochemistry of scandium and yttrium*, Part 2: biochemistry and applications. Kluwer Academic/Plenum Publishers, New York.
- Hu, Z. H., Richter, H., Sparovek, G., Schnug, E. (2004): *Physiological and biochemical effects of rare earth elements on plants and their agricultural significance: a review*. *J. Plant Nutr.*, 27: 183—220.
- Hu, Z., Haneklaus, S., Sparovek, G., Schnug, E. (2006): *Rare earth elements in soil*. *Comm. Soil Sci. Plant Anal.*, 37: 1381—1420.
- Hung Zhi-Gang, Chen Pei-Zi, Zhang Mei-Juan (1983): *Effect of spray rare earth elements on crossing early rice*. *Hunan Agri. Sci.* 4:43—49.
- Ichihashi, H., Morita, R., Tatsukawa, R. (1992): *REE in naturally grown plants in relation to their variation in soils*. *Environ Poll.* 76: 157—162.
- Jacobson, L., Overstreet, R. (1948): *The uptake by plants of plutonium and some products of nuclear fission absorbed on soil colloides*. *Soil Sci.*, 65: 129—143.
- Kabata-Pendias, A. (2001): *Trace elements in soils and plants*. Third Edition. CRC Press
- Kádár, I. (2000): *Unpublished data*.
- Kasimiva, N. S., Solncevoy, N. P., Rihklinga, A. V. (1996): *Natural raw materials: sustainable utilization and protection*. Iz. Moscow University.
- Kastori, R., Petrović, N. (1983): *The effect of excess flour on metabolic processes in plants*. *Man and the Environment*, 8: 15—19.
- Kastori, R., Petrović, N. (1984): *Sensivity of some plant species to fluorine concentrations*. *Matica srpska Proc. of Natural Sciences*. 84. 57—64.
- Klechkonsky, V. M., Tselishcheva, G. N. (1957): *Some specific features of the behavior of Sr-90/Y-90 on absorption by plants*. In: Klechkonsky, V. M. ed. *On the Behavior of Radioactive Fission Products in Soil, Their Absorption by Plants and Their Accumulation in Crop*. U.S. Atomic Energy Commission, Washington, DC. 194—221.
- Krstić, B., Stanković, Ž., Pajević, S. (1991): *Reaction of young sunflower, barley and wheat plants to different chromium concentrations in the nutrient solution*. *Zemljište i biljka*. 40: 91—97.
- Levan, A. (1945): *Cytological reactions induced by inorganic salt solutions*. *Nature*, 156: 751—753.
- Marschner, H. (1995): *Mineral nutrition of higher plants*. Academic Press. London.
- Ning Ja-Ben, Zhang Xio-Ju, Chao Zhi-Xin (1985): *Applied effect and technique of rare earth elements in sugar cane*. *Hunan Agri. Sci.* 2: 13—19.
- Nishita, H., Romney, E. M., Larson, K. H. (1961): *Upatake of radioactive fission products by crop plants*. *Agricultural and Food Chemistry*. 9: 101—106.
- Ozaki, T., Ambe, S., Enomoto, S., Minai, Y., Yoshida, S., Mekide, Y. (2002): *Multitracer study on the uptake mechanism of yttrium and rare earth elements by autumn fern*. *Radiochimica Acta*. 90, 303—307.
- Pais, I. (1983): *The biological importance of titanium*. *J. Plant Nutr.*, 6: 3—8.

- Paulenova, A., Rajec, P., Kandrak, J., Saskolova, G., Tothova, E., Bartos, P., Svec, V., Gora, R. (2000): *The study of americium, yttrium and lead complexation by humic acids of different origin*. Journal of Radioanalytical and Nuclear Chemistry, 246: 617—622.
- Picard, B. G. (1970): *Comparasion of calcium and lanthanum ions in the Avena coleoptile growth test*. Planta, 91: 314—319.
- Rediske, J. H., Selders, A.-A. (1953): *The uptake and translocation of strontium by plants*. Plant Physiol. 28: 594—605.
- Rediske, J. H., Selders, A. A. (1954): *The upatke and translocation of yttrrium by higher plants*. American J. Bot., 41: 238—242.
- Rengel, Z. (1994): *Effects of aluminium, REE and other metals on the Ca²⁺ uptake by Amaranthus protoplasts*. J. Plant Phyiol. 143: 47—51.
- Ribár, B. (2006): *Kristalne strukture itrijumovih nitrata*. Vojvođanska akademija nauka i umetnosti, Novi Sad.
- Ripan, R., Ceteanu, I. (1972): *Neorganičeskaja himija*, Tom 2, Himija metalov, Izdateljstvo Mir, Moskva.
- Robinson, W. O. (1943): *The occurence of rare earths in plants and soils*. Soil. Sci. 56: 1—6.
- Shacklette, H. T., Erdman, J. A., Harms, T. F. (1978): *Trace elements in plant foodstuffs*. In: Oehme, F. W. ed. Toxicity of Heavy Metals in the Environments, Part I. Marcel Dekker, New York.
- Solecki, J. (2004): *Yttrium-90 adsorption on various soils of successive horizons in Poland*. Journal of Radioanalytical and Nuclear Chemistry. 262: 759—766.
- Spooner, M. A. (1949): *Observations on the absorption of radioactive strontium and yttrium by marine alge*. Jour. Marine Biol. Assoc. 28: 587—625.
- Tyler, G., Olsson, T. (2001): *Plant uptake of major and minor elements as influenced by soil acidity and liming*. Plant Soil, 230: 307—321.
- Tyler, G. (2004): *Rare earth elements in soil and plant systems — a review*. Plant and Soil 267: 191—206.
- Tyler, G.(2005): *Changes in the concentrations of major, minor and rare-earth elements during leaf senescence and decomposition in Fagus sylvatica forest*. Forest Ecology and Managment. 206: 167—177.
- Tyler, G., Olsson, I. (2005): *Rare earth elements in forest-floor herbs as related to soil conditions and mineral nutrition*. Biological Trace Element Research. 106: 177—191.
- Van Steveninck, R. F., Van Steveninck, M. E., Chescoe, D. (1976): *Intercellular binding of lanthanum in root tips of barley (Hordeum vulgare)*. Protoplasma, 90:89—97.
- Welch, G. R. (1984): *Biochemical dynamics in organized states: A holistic approach*. In: Ricard, J., Cornish-Bowen, A. eds. Plenum Press, New York, 85—101.
- Xie Hui-Guang, Yu Zheng-He (1986): *Research of effect of rare earth elements on quantities and quality of suagr beet*. China Sugar Beet J. 1:18—24.
- Yang, L. H., Wang, X. R., Sun, H. (1999): *The effect of EDTA on rare earth elements bioavailability in soil ecosystem*. Chemosphere, 38: 2825—2833.
- Young, R. R. (1935): *Certain rarer elements in soil and fertilizers and their role in plant growth*. Mem. Cornell Agric. Exp. Stan. 174: 1—70.

ЕЛЕМЕНТИ РЕТКИХ ЗЕМАЉА — ИТРИЈУМ И ВИШЕ БИЉКЕ

Рудолф Р. Кастори¹, Ивана В. Максимовић¹, Тијана М. Зеремски²,
Марина И. Путник-Делић¹

¹ Пољопривредни факултет, Трг Доситеја Обрадовића 8,
21000 Нови Сад, Србија

² Институт за ратарство и повртарство, Максима Горког 30,
21000 Нови Сад, Србија

Резиме

Елементи ретких земаља (REEs) су из хемијски униформних група са веома сличним физичким и хемијским особинама. REEs укључују елементе као што су: скандијум, итријум и лантаниде од лантана до лутецијума. Они су широко распрострањени и присутни у свим деловима биосфере. REEs се користе у индустрији, пољопривреди, медицини, биотехнологији, код проблема заштите животне средине и у многим другим пољима. У новије време, у многобројним огледима је утврђено њихово позитивно или негативно, пре свега неспецифично, дејство на животне процесе виших биљака као и на растење и принос гајених врста, али физиолошки механизми дејства још увек нису разјашњени. Утврђено је да је итријум широко распрострањен у биљном свету, као и да поједине биљне врсте итријум у различитој мери усвајају. Највеће је његово накопљање у корену и листовима. Иако је итријум откривен пре више од два века, о његовом дејству на више биљке, на њихову анатомску и морфолошку грађу, физиолошке и биохемијске процесе и др. веома мало је познато. Један од основних разлога за то је да итријум, као и остали елементи REEs према досадашњим сазнањима нису биогени за више биљке и шире за живе организме. Циљ овог прегледа је да у сажетом облику прикаже досадашња сазнања у вези са итријумом у биљном свету.

*Dejana M. Džigurski¹, Slobodanka J. Stojanović¹,
Aleksa S. Knežević¹, Ljiljana M. Nikolić¹,
Branka B. Ljevnaić-Mašić¹*

¹ University of Novi Sad, Faculty of Agriculture,
Trg D. Obradovica 8, 21000 Novi Sad, Serbia

VEGETATION OF THE CLASSES *HYDROCHARI-LEMNETEA* OBERD. 1967 AND *POTAMETEA* TX. ET PRSG. 1942 IN THE JEGRIČKA WATERCOURSE (THE PROVINCE OF VOJVODINA, SERBIA)

ABSTRACT: The Jegrička, once a natural watercourse traversing the southwestern part of the Bačka region, has been turned into a canal, which became part of the main canal network of the hydro-system Danube-Tisza-Danube (Hs DTD). In its turn, the Hs DTD is part of the European waterway linking the North Sea to the Black Sea, i.e., part of the navigable Rhine-Main-Danube Canal. The watercourse is 65.4 km long and it is divided into three levels. The presence of the regulated and the nonregulated sections of the canal, frequent and abrupt changes in water level in the individual sections, different depths and surface water widths of the various sections and the fishpond constructed in the lower section cause considerable vegetation diversity. The vegetation comprises aquatic associations of the classes *Hydrochari-Lemnetea* Oberd. 1967 and *Potametea* Tx. et Prsg. 1942. The class *Hydrochari-Lemnetea* Oberd. 1967 includes the following phytocoenoses: *Salvinio-Spirodeletum polyrrhizae* Slavnić 1956, *Ceratophylletum demersi* (Soó 27) Hild. 1956, *Lemno-Utricularietum vulgaris* Soó 1928 and *Hydrocharidetum morsus-ranae* Van Langendonck 1935. The class *Potametea* Tx. et Prsg. 1942 includes the associations *Myriophyllo-Potametum* Soó 1934, *Najadetum marine* Fukarek 1961, *Nymphaetum albae* Vollmar 1947, *Nymphoidetum peltate* (Allorge 1922) Oberd. et Müller 1960 and *Trapetum natantis* Müller et Görs 1960.

KEY WORDS: Jegrička, aquatic vegetation, floating vegetation, submerged vegetation

INTRODUCTION

The Jegrička watercourse is a regulated canal that is part of the main canal network of the hydro system Danube-Tisza-Danube (Hs DTD). It is located in the Province of Vojvodina, Serbia. It runs from the village of Despotovo to the village of Žabalj. Its length is 65.4 km and its drainage basin covers 60,380 ha. The canal is divided into three levels: the upper, from the Despotovo lock (km 64+163) to the Zmajevo weir (km 47+700), the central,

from the Zmajevu weir to the Žabalj II lock (km 15+000), and the lower, from the Žabalj II lock to the Žabalj pump station and the confluence with the Tisza River (km 0+000). The canal has a regulated bed between the marks km 64+163 and km 30+000, and a nonregulated bed from the mark km 30+000 to the confluence with the Tisza River. The lower section of the canal has been turned into a fishpond, the area of which is about 283 ha while the width reaches up to 200 m. Because of the controlled water level table, regulated and nonregulated sections, and the fishpond in the lower section, the watercourse is very specific from the ecological point.

The following villages are arranged around the Jegrička watercourse from its source to the mouth: Despotovo, Ravno Selo, Zmajevu, Sirig, Temerin, Gospođinci, and Žabalj.

The earlier studies of the aquatic and semiaquatic vegetations of the Jegrička watercourse have covered a limited area of the central section (Vučković et al., 1993; Stojanović et al., 1996). The results of this study cover the aquatic phytocoenoses of the classes *Hydrochari-Lemnetea* Oberd. 1967 and *Potametea* Tx. et Prsg. 1942. and it is part of complex, long-term investigation of the total length of the Jegrička watercourse (Lazić, 2003, 2006; Stojanović et al., 2007).

MATERIAL AND METHODS

The phytocoenological studies were conducted during 1997–2004 vegetation periods according to the principles of the Zürich-Montpellier School (Braun-Blanquet, 1964). The syntaxonomic review of the studied vegetation was done according to Soó (1964–1980). Plant determination was done according to the publications *Flora of SR Serbia, I–IX* (Josifović, ed. 1970–1977), *Flora of SR Serbia, X* (Sarić, ed. 1986), *Flora of Serbia, I* (Sarić, 1992), *Flora Europaeae, I–V* (Tutin et al., 1964–1980 I–V), *Hinár határozó* (Felföldy, 1990), and *Icanographie der Flora des Südostlichen Mitteleuropa* (Jávorka & Csapody, 1975).

RESULTS AND DISCUSSION

Syntaxonomic review of the associations of classes *Hydrochari-Lemnetea* and *Potametea* of the watercourse Jegrička:

Class: *HYDROCHARI-LEMNETEA* Oberd. 1967

Order: *Hydrocharietalia* Rübel 1933

Alliance: *Lemnon minoris* W. Koch et Tx. ex Oberd 1957

1. Ass. *Salvinio-Spirodeletum polyrrhizae* Slavnić 1956

Alliance: *Ceratophyllion* Den Hartog et Segal 1964

2. Ass. *Ceratophylletum demersi* (Soó 27) Hild. 1956

subass. *typicum*

subass. *hydrocharetosum morsus-ranae* Lazić 2003

Alliance: *Hydrocharition* Rübel 1933

3. Ass. *Lemno-Utricularietum vulgaris* Soó 1928

4. Ass. *Hydrocharidetum morsus-ranae* Van Langendonck 1935

Class: *POTAMETEA* Tx. et Prsg. 1942

Order: *Potametalia* W. Koch 1926

Alliance: *Potamion* W. Koch 1926 emend. Oberd. 1957

5. Ass. *Myriophyllo-Potametum* Soó 1934

subass. *typicum*

subass. *potametosum crispum* Slavnić 1956

6. Ass. *Najadetum marine* Fukarek 1961

Alliance: *Nymphaeion* Oberd. 1956

7. Ass. *Nymphaeetum albae* Vollmar 1947

8. Ass. *Nymphoidetum peltate* (Allorge 1922) Oberd. et Müller 1960

9. Ass. *Trapetum natantis* Müller et Görs 1960

1. Ass. *Salvinio-Spirodeletum polyrrhizae* Slavnić 1956 (Tab. 1, col. 1)

Tab. 1 — *Lemnion minoris*, *Ceratophyllion* and *Hydrocharition*

	1	2a	2b	3	4
Characteristic species of the ass. and subass. of the alliances <i>Lemnion minoris</i>, <i>Ceratophyllion</i> and <i>Hydrocharition</i>					
<i>Salvinia natans</i> (L.) Allioni	V ₊₃		I ₁	II ₊	II ₊
<i>Spirodela polyrrhiza</i> (L.) Schleid.	V ₁₋₃		I ₁	II ₊	I ₊
<i>Ceratophyllum demersum</i> L.	V ₊₂	V ₄₋₅	V ₃₋₄	V ₊₁	V ₊₂
<i>Hydrocharis morsus-ranae</i> L.	V ₊₂		V ₁₋₂	III ₊₁	V ₃₋₄
<i>Utricularia vulgaris</i> L.		I ₁		V ₃₋₄	I ₊
<i>Lemna minor</i> L.	V ₊₂		I ₊₂	III ₊₁	I ₊
Characteristic species of the alliances <i>Lemnion minoris</i>, <i>Ceratophyllion</i> and <i>Hydrocharition</i>, the order <i>Hydrocharietalia</i> and the class <i>Hydrochari-Lemnetae</i>					
<i>Vallisneria spiralis</i> L.	II ₊₁		I ₊		II ₁₋₂
<i>Lemna gibba</i> L.	III ₊				
<i>Myriophyllum spicatum</i> L.	I ₊	IV ₊₁	III ₊	IV ₊₃	IV ₊₁
<i>Potamogeton fluitans</i> Roth.			I ₂		
<i>Najas marina</i> L.		I ₊			
Accessory species					
<i>Trapa natans</i> L.	II ₊		I ₁₋₂	II ₊	III ₊₁
<i>Elodea canadensis</i> Rich	II ₊	I ₊			II ₊₂
<i>Potamogeton perfoliatus</i> L.			I ₁		
<i>Polygonum amphibium</i> L.	I ₊		I ₊		I ₊
<i>Nymphaea alba</i> L.		I ₊		II ₁	
<i>Phragmites communis</i> Trin.				III ₁	
<i>Typha angustifolia</i> L.				II ₁	
<i>Lycopus europeus</i> L.				III ₊	
<i>Sparganium ramosum</i> Huds.				II ₁	
<i>Glyceria maxima</i> (Hartm.) Holm.				II ₁	
<i>Rumex hydrolapathum</i> Huds.				II ₁	

1 — *Salvinio-Spirodeletum polyrrhizae*; 2a — *Ceratophylletum demersi* subass. *typicum*;

2b — *Ceratophylletum demersi* subass. *hydrocharetosum*; 3 — *Lemno-Utricularietum vulgaris*;

4 — *Hydrocharidetum morsus-ranae*

In the studied watercourse, thick stands of the association *Salvinio-Spirodeletum polyrrhizae* were found near the Despotovo lock. Sparse stands were found adjoining reed stands in the locations of Temerin and Sirig. Under conditions of intensive light, these stands become abundant in warm sites with sluggish water flow. The main reasons for a limited distribution of these stands are the abrupt and frequent changes in water level and flow rate. Optimum growth is achieved in the second half of summer, when *Salvinia natans*, a characteristic species of the association, reaches maximum abundance and sociability. In addition to the characteristic species *Salvinia natans* and *Spirodela polyrrhiza*, the floristic structure of the association frequently includes the species *Hydrocharis morsus-ranae*, *Lemna minor* and *Ceratophyllum demersum*.

2. Ass. *Ceratophylletum demersi* (Soó 27) Hild. 1956 (Tab. 1, col. 2a, 2b)

The phytocoenosis *Ceratophylletum demersi* has differentiated in the studied watercourse into subassociations *Ceratophylletum demersi* subass. *typicum* and *Ceratophylletum demersi* subass. *hydrocharetosum morsus-ranae*. Stands of *Ceratophylletum demersi* (Soó 27) Hild. subass. *typicum* (Tab. 1, col. 2a) are widely distributed in the lower, nonregulated section of the Jegrička, while their distribution is limited in the upper and central sections. These stands occupy deep parts in the middle of the stream. Enormous submersed stands of the dominant species *Ceratophyllum demersum*, which are either monodominant or floristically poor, can be seen near the village of Žabalj and in the Žabalj fishpond. Stands of *Ceratophylletum demersi* (Soó 27) Hild. 1956 subass. *hydrocharetosum morsus-ranae* Lazić 2003 (Tab. 1, col. 2b) develop in sluggish water closer to the banks. They can be seen in the locations of Temerin, Gospodinci, Sirig, Ravno Selo, Žabalj, and the Žabalj fishpond. These stands are floristically richer but they cover smaller areas than those of the previous subassociation. Besides the submersed species *Ceratophyllum demersum* and *Myriophyllum spicatum*, these stands include also some floating macrophytes among which the dominant one is *Hydrocharis morsus-ranae*.

3. Ass. *Lemno-Utricularietum vulgaris* Soó 1928 (Tab. 1, col. 3)

Stands of the phytocoenosis *Lemno-Utricularietum vulgaris* were registered near the villages of Temerin and Žabalj. Shallow waters next to the belt of cattails and reeds or where the belt is thin or interrupted provide optimum ecological conditions for these stands. The layer of submersed plants includes *Utricularia vulgaris* as the dominant species and *Ceratophyllum demersum* and *Myriophyllum spicatum*, which occur in great abundance. In the layer of floating plants, the characteristic species *Lemna minor* is accompanied by *Hydrocharis morsus-ranae*, which is present in significant numbers. Since the stands of this phytocoenosis are in contact with the emerged vegetation, their floristic composition includes the species *Phragmites communis*, *Typha angustifolia*, *Sparganium ramosum*, *Lycopus europeus*, etc.

4. Ass. *Hydrocharidetum morsus-ranae* Van Langendonck 1935 (Tab. 1, col. 4)

Stands of ass. *Hydrocharidetum morsus-ranae* develop in sluggish, shallow, and warm waters in the locations of Zmajevu, Temerin and Sirig. These

stands border the submersed ass. *Ceratophylletum demersi* towards the middle of the watercourse and the emerged phytocoenoses *Scirpo-Phragmitetum*, *Typhetum angustifoliae* and *Glycerietum maximae* towards the shore. Large numbers of the characteristic species of the association, *Hydrocharis morsus-ranae*, were present in all of the analyzed stands. Among the characteristic species of the alliance, order and class, *Ceratophyllum demersum* and *Myriophyllum spicatum* were distinguished for maximum abundance, percent cover and sociability.

Tab. 2 — *Potamion* and *Nymphaeion*

	1a	1b	2	3	4	5
Characteristic species of the ass. and subass. of the alliances <i>Potamion</i> and <i>Nymphaeion</i>						
<i>Myriophyllum spicatum</i> L.	V ₁₋₂	V ₁₋₃	I ₊	IV ₊₃	II ₁	III ₊
<i>Potamogeton fluitans</i> Roth.	V ₃₋₄					
<i>Potamogeton perfoliatus</i> L.	III ₁₋₂			I ₊		
<i>Potamogeton crispus</i> L.		V ₃₋₄				
<i>Najas marina</i> L.			V ₃₋₅	I ₊	II ₊₁	
<i>Nymphaea alba</i> L.				V ₃₋₅	I ₁	
<i>Nymphoides flava</i> Hill.			II ₊₁	I ₁	V ₄₋₅	
<i>Trapa natans</i> L.			I ₊	I ₊	I ₊	V ₃₋₄
Characteristic species of the alliances <i>Potamion</i> and <i>Nymphaeion</i>, the order <i>Potametalia</i> and the class <i>Potametea</i>						
<i>Ceratophyllum demersum</i> L.	IV ₁₋₂	V ₊	V ₊₂	IV ₊₃	IV ₊₂	V ₊₃
<i>Spirodela polyrrhiza</i> (L.) Schleid.			I ₊	I ₊	II ₊	I ₊
<i>Hydrocharis morsus-ranae</i> L.		V ₊₁		I ₊	I ₊	V ₊₃
<i>Utricularia vulgaris</i> L.				I ₁₋₃	I ₊	
<i>Lemna minor</i> L.			I ₊	I ₊	II ₊	I ₊
<i>Lemna trisulca</i> L.			I ₊		II ₊₂	
<i>Salvinia natans</i> (L.) Allioni	I ₊	I ₊₁				II ₊
<i>Vallisneria spiralis</i> L.						I ₊
Accessory species						
<i>Butomus umbellatus</i>	II ₊₁					
<i>Polygonum amphibium</i> L.		I ₁		I ₊		I ₊
<i>Elodea canadensis</i> Rich						I ₂
<i>Juncus compressus</i> Jacq.						I ₊
<i>Carex vulpina</i> L.						I ₊

1a — *Myriophyllo-Potametum* subass. *typicum*; 1b — *Myriophyllo-Potametum* subass. *potametosum crispum*; 2 — *Najadetum marine*; 3 — *Nymphaeetum albae*; 4 — *Nymphoidetum peltate*; 5 — *Trapetum natantis*

5. Ass. *Myriophyllo-Potametum* Soó 1934 (Tab. 2, col. 1a, 1b).

In the Jegrička watercourse, the association *Myriophyllo-Potametum* has differentiated into subassociations *Myriophyllo-Potametum* subass. *typicum* and *Myriophyllo-Potametum* subass. *potametosum crispum*. ***Myriophyllo-Potametum* Soó 1934 subass. *typicum*** (Tab. 2, col. 1a) grows only in the vicinity of Ravno Selo. It is floristically very poor, adjoining in the ecological sequence stands of ass. *Ceratophylletum demersi*. Stands of ***Myriophyllo-Potametum* Soó 1934 subass. *potametosum crispum*** Slavnić 1956 (Tab. 2, col. 1b) found fa-

avorable conditions for development in the location of Temerin, in an inlet where the water is warm and hardly moving. Besides *Potamogeton crispus*, the characteristic species of the association whose abundance and percentage cover are enormous, the species *Myriophyllum spicatum*, *Ceratophyllum demersum*, *Hydrocharis morsus-ranae* and *Polygonum amphibium* are present in significant numbers.

6. Ass. *Najadetum marine* Fukarek 1961 (Tab. 2, col. 2)

Stands of ass. *Najadetum marine* develop in deep waters of the Žabalj fishpond, several meters away from the banks. The entire right bank of the fishpond, which is bordered by saline pastures on the solonchak soil, is overgrown with thick, monodominant stands of the association which are scores of meters long and several meters wide. On the left bank, which adjoins agricultural fields of different soil types, the calcareous chernozem, hydromorphic smonitza soil and alluvial deposits on the hydromorphic black soil, the stands of the association are patchy, small in size and they are predominated by *Najas marina*, which is sparsely intermixed with *Ceratophyllum demersum*, *Nymphoides flava*, *Trapa natans*, *Myriophyllum spicatum*, *Lemna minor*, *L. trisulca* and *Spirodela polyrrhiza*.

7. Ass. *Nymphaeetum albae* Vollmar 1947 (Tab. 2, col. 3)

Large stands of the phytocoenosis *Nymphaeetum albae* grow in the locations of Žabalj, Gospodinci and Temerin and in the Žabalj fishpond, i.e., in the sections with nonregulated bed where the water flow is slow and the thermal and light regimens are favorable. The floristic structure of the association is predominated by the species *Nymphaea alba* L., which has high values of abundance, percent cover and sociability in all stands. The species *Ceratophyllum demersum* and *Myriophyllum spicatum* are present in considerable numbers in these stands.

8. Ass. *Nymphoidetum peltate* (Allorge 1922) Oberd. et Müller 1960 (Tab. 2, col. 4)

Stands of the association *Nymphoidetum peltate* adjoin the stands of the phytocoenosis *Nymphaeetum albae*. In the location of Žabalj and in the Žabalj fishpond, the former stands form belts of floating vegetation, which find optimum conditions in the shallow, sluggish, warm waters rich in nutrients. Besides the dominant species *Nymphoides flava*, the species *Nymphaea alba*, *Hydrocharis morsus-ranae*, *Trapa natans*, *Spirodela polyrrhiza* and *Lemna minor* sporadically provide the characteristic physiognomy to the floating layer. The species *Ceratophyllum demersum*, *Myriophyllum spicatum*, *Lemna trisulca*, *Najas marina* and *Utricularia vulgaris* make a clearly differentiated submersed layer.

9. Ass. *Trapetum natantis* Müller et Görs 1960 (Tab. 2, col. 5)

The association *Trapetum natantis* is predominant in the floating belt of vegetation in the locations of Ravno Selo, Zmajevo, Sirig and Temerin. The association has a limited distribution in the Žabalj fishpond. The considerable

percent cover and floristic poverty of its stands are due to the abundance of the dominant species *Trapa natans*, which restricts the development of other aquatic plants. When stands of ass. *Trapetum natantis* adjoin stands of ass. *Hydrocharidetum morsus-ranae* and ass. *Ceratophylletum demersi*, the species *Hydrocharis morsus-ranae*, *Salvinia natans*, *Polygonum amphibium*, *Spirodela polyrrhiza* and *Lemna minor* can be seen in their floating layer and the species *Ceratophyllum demersum*, *Myriophyllum spicatum*, *Elodea canadensis* and *Valisneria spiralis* can be found in their submersed layer.

REFERENCES

- Braun-Blanquet, J. (1964): *Pflanzensoziologie*. Wien—New York.
- Felföldy, L. (1990): *Visugyi hidrobiologia*. 18-Kötet-Hinar haterarazo Korniezet-veldelmi es Teruletfejlesztési Miniszterium, Budapest, 1—144.
- Jávorka, S., Csapody, V. (1975): *Icanographie der Flora des Südostlichen Mitteleuropa*. Akadémiai Kiado, Budapest.
- Josifović, M. (ed.) (1970—1977): *Flora of SR Serbia*, I—IX, SANU, Beograd.
- Kilibarda, P. (1996): *Jegrička kao značajno prirodno dobro*. Edicija Tija voda, Monografija 1, Novi Sad, PČESA, 154—159.
- Lazić, D. (2003): *Floristic-phytocoenological study of the flora of the Jegrička stream*. M. Sc. thesis, University of Novi Sad, Faculty of Agriculture, Novi Sad, 1—135.
- Lazić, D. (2006): *Vaskularna flora i vegetacija OKM Hs DTD na području Bačke — stanje i uticaj na korišćenje i održavanje*. Doktorska disertacija, PMF, Univerzitet u Novom Sadu, 1—198.
- Sarić, M. (ed.) (1986): *Flora of SR Serbia X*. SANU, Beograd.
- Sarić, M. (ed.) (1992): *Flora of SR Serbia I*. SANU, Beograd.
- Soó, R. (1964—1980): *A magyar flóra és vegetáció rendszertani-növényföldrajzi kézikönyve I—VI*. Akadémiai kiadó, Budapest.
- Stojanović, S., Vučković, M., Stanković, Ž., Žderić, M., Kilibarda, P. (1996): *O flori i vegetaciji Jegričke*. Monografija Jegrička, Edicija Tija voda, PČESA, Novi Sad, 61—67.
- Stojanović, S., Lazić, D., Knežević, A., Nikolić, Lj., Škorić, M., Kilibarda, P., Mišković, M., Bugarski, R. (2007): *Flora i vegetacija OKM HS DTD u Bačkoj*. Univerzitet u Novom Sadu, Poljoprivredni fakultet, JVP „Vode Vojvodine”, Novi Sad, 1—204.
- Tutin, T. G., Heywood, V. H., Burges, N. A., Valentine, D. H., Walters, S. M., Webb, D. A. eds. (1964—1980): *Flora Europaea I—V*. Cambridge University press, Cambridge.
- Vučković, M., Stojanović, S., Stanković, Ž., Žderić, M., Kilibarda, P., Radak, Lj. (1993): *Aquatic and march vegetation of Jegrička*. Review of research Faculty of Sciences, biology series, University of Novi Sad, Institute of Biology, 23: 75—78.

ВЕГЕТАЦИЈА КЛАСА *HYDROCHARI-LEMNETEA* OBERD. 1967.
И *POTAMETEA* TX. ET PRSG. 1942. У ВОДОТОКУ ЈЕГРИЧКЕ
(ВОЈВОДИНА, СРБИЈА)

Дејана М. Цигурски, Слободанка Ј. Стојановић, Алекса С. Кнежевић,
Љиљана М. Николић, Бранка Б. Љевнаић-Машић¹

¹ Универзитет у Новом Саду, Пољопривредни факултет,
Трг Д. Обрадовића 8, Нови Сад, Србија

Резиме

Јегричка, некада природни водоток југозападнoг дела Бачке, каналисана је и прикључена ОКМ Хс ДТД, који је део трансверзалног европског пловног пута од Северног до Црног мора. Њен ток дужине 65,4 km чине три степенаста база-на. Присуство деоница са регулисаним и нерегулисаним коритом, честе и нагле промене нивоа воде у појединим базенима, различита дубина и ширина воденог огледала и формирани рибњак, условљавају знатну вегетацијску разноликост. Вегетацију Јегричке чине заједнице класа *Hydrochari-Lemnetea* и *Potametea*. Из класе *Hydrochari-Lemnetea* констатоване су фитоценозе: *Salvinio-Spirodeletum polyrrhizae*, *Ceratophylletum demersi*, *Lemno-Utricularietum vulgaris* и *Hydrocharidetum morsus-ranae*. Из класе *Potametea* присутне су састојине асоцијација: *Myriophyllo-Potametum*, *Najadetum marine*, *Nymphaeetum albae*, *Nymphoidetum peltate* и *Trape-tum natantis*.

*Ivica G. Đalović,¹ Ivana V. Maksimović,²
Rudolf R. Kastori,² Miodrag Ž. Jelić³*

¹ Institute of Field and Vegetable Crops, Novi Sad, 21000 Novi Sad, Serbia

² Faculty of Agriculture Novi Sad, Trg D. Obradovića 8, 21000 Novi Sad, Serbia

³ Faculty of Agriculture Zubin Potok, Jelene Anžuske bb, 38228, Zubin Potok, Serbia

MECHANISMS OF ADAPTATION OF SMALL GRAINS TO SOIL ACIDITY*

ABSTRACT: Acid soils limit crop production on 30—40% of the world's arable land and up to 70% of the world's potentially arable land. Over 60% of the total arable lands in Serbia are acid soils. Soil acidity is determined by hydrogen (H^+) in soil solution and it is influenced by edaphic, climatic, and biological factors. Major constraints for plant growth on acid mineral soils are toxic concentrations of mineral elements like Al of H^+ and/or low mineral nutrient availability due to low solubility (e.g. P and Mo) or low reserves and impaired uptake (e.g. Mg^{2+}) at high H^+ concentrations. Aluminum (Al) toxicity is primary factor limiting crop production on acid soils. This review examines our current understanding of mechanisms of Al-toxicity, as well as the physiological and genetic basis for Al-toxicity and tolerance. Inhibition of root growth by Al leads to more shallow root systems, which may affect the capacity for mineral nutrient acquisition and increase the risk of drought stress. Of the two principal strategies (tolerance and avoidance) of plants for adaptation to adverse soil conditions, the strategy of avoidance is more common for adaptation to acid mineral soils. At the same, the short view of the most important genetics tolerance mechanisms, developed and determined in some small grains genotypes, is showed as well.

KEY WORDS: acid soils, adaptation mechanisms, small grains

INTRODUCTION

Acid soils currently reduce plant production on 30—40% of total and up to 70% of potentially arable land worldwide. They are distributed mainly in two distinctive geographical belts: in the cold, humid, and moderate climate of a northern belt, and the warm and humid climate of southern tropical regions (Eswaran et al., 1997).

Van Wambeke (1976) reported that acid soils cover 1,455 million hectares of land, or some 11% of total global land surface, while Von Uexkull and Mutert (1995) made estimates that acid soils (defined as soils

* Review paper.

with $\text{pH} < 5.5$ in the top layer) cover 3,950 million hectares, or about 30% of arable land worldwide, with a tendency of further growth.

In the Republic of Serbia, acid soils are widespread, accounting for over 60% of total arable land (Stevanović et al., 1995). Those are mostly lowland or hillside types of pseudogley or its leached variants, acid vertisols, podzolic eutric cambisols, diluvial, brown, or leached brown soils of mountainous regions. Those soils are rather poor in bases, medium to heavily acidic, having very poor texture and poor organic content, and more or less ill suited for cultivation of most cereal crops. Most acid soils are located in the central parts of Serbia and in Kosovo and Metohija. With the exception of soils in major river valleys (formed upon alluvial deposits) and soils formed upon calcareous, marine and lake sediments and limestone, nearly all regions of central Serbia have soils with some degree of acidity.

The acidity of these soils, their high contents of H^+ ions and low contents of essential plant nutrients, primarily P and Ca, are limiting factors for high and stable yields of cultivated cereal crops. Apart from acidity, those soils are also often characterized by high contents of toxic forms of Al, Fe and Mn, and by deficits caused by leaching or decreased availability of P, Ca, Mg and some other micronutrients, especially Mo, Zn and B (Narro et al., 2001; Sumner, 2004; Welcker et al., 2005; Kovačević et al., 2006; Jovanović et al., 2006; Đalović et al., 2007).

Recent investigations worldwide have shown that massive deterioration of small grains on acid soils is caused by elevated concentrations of mobile forms of some toxic elements (Al, Fe, Mn), whose contents become especially evident when no phosphorus nutrition is practiced or the Ca component is missing from nitrogen fertilizers (Jelić, 1996).

Acidity restrains root growth and, consequently, the uptake of water and mineral nutrients. The top soil layer containing more organic matter is dominated by H^+ ions, while Al toxicity is more evident in layers beneath.

Aluminum toxicity is considered to be the most important factor limiting plant growth on acid soils (Foy, 1984; Carver and Ownby, 1995; Jayasundara et al., 1998; Arsenijević-Maksimović et al., 2001; Jelić et al., 2004). Even though Al is present in water, soil, and atmosphere, most of it is bound to aluminosilicate minerals in soil, while small, submicromolar amounts appear as soluble forms able to affect biological systems (May and Nordstrom, 1991).

When their contents and mobility are high in soil, plants become stressed, and the state of stress becomes evident from such negative symptoms as slow growth, weak tillering, thinning of crop and delay in various stages of development. High Al concentrations in plants interfere with the uptake, transport, and reutilization of calcium, magnesium, phosphorus, and potassium that coincide with water uptake and enzymatic activity in the root system, and disrupt plant homeostasis. Obstruction of development of generative organs and grain filling, and frequent deterioration and death of plants, are the other consequences of the toxic activity of Al.

A primary response to Al-induced stress occurs in the root system of plants (Taylor, 1988; Jayasundara et al., 1998). The main symptom

of Al toxicity is fast inhibition of root growth, which may be caused by changes in various physiological processes, including Al interaction with other ions in the cell wall, plasma membrane or symplast of the root system (Marschner, 1991; Horst, 1995; Kochian, 1995). Aluminum also impairs cell division in root tips, hardens cell walls, interferes with DNA replication, blocks a number of enzymes, reduces the production and transport of cytokinin, modifies the structure and functioning of plasma membrane, binds phosphorus in its less available forms in soil and on root surface, decreases root respiration, reduces water uptake and blocks the uptake and metabolism of most nutrients.

Plant species differ regarding their tolerance to soil acidity and high content of mobile Al in the nutrient media. Some of them are intrinsically more tolerant than others, for example cowpea (*Vigna unguiculata* L. Walp), peanut (*Arachis hypogea* L.), rice (*Oryza sativa* L.) or rye (*Secale cereale* L.) (Little, 1988). Rye is one of the most stress-tolerant species in Triticeae family. Hitherto research of the tolerance of cereal species has shown that rye is most tolerant, followed by triticale (*X Triticosecale* Wittmack), wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) (Aniol and Madej, 1996).

We intended to discuss mechanisms of adaptation of small grains to soil acidity, focusing on high contents of mobile Al in nutrient medium. Acid soils are in a process of world scale expansion, largely due to human activities, and this issue has scientific as well as acute economic relevance.

MECHANISMS OF SMALL GRAINS ADAPTATION TO SOIL ACIDITY AND HIGH ALUMINIUM CONTENTS

Adaptation of small grains to elevated soil acidity and high Al contents in soil solution may be due to one or more different mechanisms (Marschner, 1991). Our present knowledge shows that various types of mechanisms, depending on their sites of activity, can be grouped into: *i*) external mechanisms that stimulate Al elimination from root tips; *ii*) internal mechanisms dependent on the capacity of Al tolerance in plant symplast, and *iii*) genetic mechanisms. One should bear in mind that soil acidity normally coincides with high Al contents, but that some plant species are sensitive to acidity and at the same time tolerant to high Al concentration, while the reverse applies to some other species.

External mechanisms of tolerance

External mechanisms of tolerance act to prevent penetration of Al and its accumulation in plants. Their activity is present in the apoplast, cell wall, and rhizosphere. The activity of external mechanisms of tolerance is based on: Al immobilization in cell wall (Blamey et al., 1990; Taylor, 1991; Kochian, 1995), leaching of Al through plasma membrane (Zhang and Taylor, 1991; Taylor, 1991), formation of a pH barrier in the plant rhizosphere (Foy, 1988; Taylor, 1991; Pellet et al., 1997) and secretion of

organic acids, phosphates, chelates and the other ligands through the root to the exterior (Henderson and Ownby, 1991; Ryan et al., 1995; De la Fuente et al., 1997; Pellet et al., 1997).

Cell wall is a barrier for Al ions transported from nutrient medium into the cell interior. For that reason, cell wall, together with plasma membrane, provides the most important external mechanism of plant protection from the toxic effects of Al (Takabatake and Shimmen, 1997). Complex structure of the cell wall and its chemical composition provides a protection from abiotic stress, including Al-induced stress. Evidence has been found that different wheat cultivars have different capacities to bind Al in the cell wall, consequently demonstrating different levels of Al tolerance. However, mechanisms of tolerance of some genotypes are still not clear.

Plasma membrane is the primary site where Al intoxication takes place (Takabatake and Shimmen, 1997). Primary effects of Al on the permeability of root membrane may appear several minutes or as much as several hours after exposure to Al. These effects are probably due to an ability of Al to bind carboxyl groups in the cell wall and phosphate groups in the membrane (Gunsé et al., 1997). Although the first reaction to Al is taking place at the tip of the root (Taylor, 1995; Sivaguru et al., 1999), the mechanism of growth inhibition caused by Al has still not been adequately explained and continues to provoke different opinions. Some data indicate that Al penetrates the root symplast at considerable degree, probably affecting membrane growth (Lazof et al., 1994).

As the exact target site of Al toxicity in the cell has not yet been identified, intensive research has focused on the symplast on one side, and the apoplast on the other (Marienfeld et al., 2000). The bulk of absorbed Al accumulates in the apoplast and accounts for 30–90% of total Al in tissues (Rengel, 1996). Most of Al has thus been uncritically attributed to symplast as a result either of apoplast contamination or insufficient desorption. Although various research reports have indicated that Al binds to different cell components, such as cell wall, plasma membrane or DNA (Rengel, 1996; Silva et al., 2000; Taylor et al., 2000), it seems that Al mostly accumulates in the cell wall. Rengel and Reid (1997) used giant cells of *Chara corallina* algae and found that 99.99% of total Al in those cells was accumulating in cell wall, while Chang et al. (1999) found that this applied mostly to the cell wall pectin that remains in the protoplast even after enzyme digestion of the wall. The authors went so far to assume that Al is able to bind pectin formed after Al treatment. However, in order to understand the mechanism of Al tolerance, quantitative information is required to clarify the uptake and distribution of Al inside the cell.

During contact with plasma membrane, Al acts as an ion-exchanging agent. Therefore, cation exchange capacity (CEC) is very important for its uptake, as hitherto research has confirmed that Al-tolerant genotypes have significantly lower CECs than Al-sensitive genotypes. Besides, higher Al accumulation in sensitive wheat and barley cultivars than in tolerant cultivars has also been found inside the root and on total root surface, while tolerant cultivars had higher Al accumulation only in the root cap zone (Foy, 1988).

Wagatsuma (1983) also found a positive correlation between CEC and Al content in roots of some plant species. However, other data based on Al distribution between wheat apoplast and symplast have not indicated any genotype differences regarding Al tolerance. Al immobilization in plasma membranes is also based on maintenance of its selective permeability. Specific calmodulin-type proteins have a significant role in this process as they are intensively synthesized by tolerant cultivars when plants are exposed to Al stress.

Aluminum phytotoxicity to cereals is heavily dependent on soil pH as well. The mechanism of Al tolerance by some cereal genotypes is based on their ability to maintain high rhizosphere pH. So far, it has been shown that tolerant cultivars have higher rhizosphere pH than Al-sensitive ones (Foy and Fleming, 1982). Wagatsuma and Yamasaku (1985) discovered a positive correlation between Al tolerance of barley and increased pH in its medium caused by the activity of the root system. However, in trials involving different nitrogen forms ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$), the build-up of a pH barrier in order to achieve wheat tolerance was less important than some other mechanisms and was very slow under conditions described (Taylor, 1988). On the other hand, there is information indicating that increased pH aiming at establishing a pH barrier is highly efficient despite being quite slow.

Secretion of organic acids, phosphates and other substances by the root system is another crucial mechanism of tolerance to Al. The activity of this mechanism is based on complexation of Al with mucilage, organic acid ligands, and other substances forming chelate complexes. Al complexation reduces its uptake from the rhizosphere of the root system. At low soil pH and high Al concentration, tolerant genotypes increase secretion of mucilage, i.e. substances characterized by a high capacity of binding Al. Those substances are able to bind cations (primarily Ca^{2+}) and are secreted mostly by the root cap and the root tip zone (Puthota et al., 1991). Under high Al content in a soil solution, the tolerant wheat cultivar Atlas 66 was found to have three times the volume of mucilage synthesis that the sensitive cultivar Victor had (Johnson and Bennet, 1991).

Aluminum complexation with organic acids reduces Al phytotoxicity in the rhizosphere or the root system. Organic acids extracted from roots have different capacities for precipitating Al, namely: oxalic acid > citric acid > malic acid > succinic acid. Delhaize et al. (1993) reported that the release of apple acid from the root tip zone of tolerant genotypes was 5–10 times higher than that of Al-sensitive genotypes. Simultaneously, accumulation of Al in the root tip zone decreased.

Tolerant plants also counter toxic activity of Al by secreting chelates by the root system. Chelators on root surface or in cell walls bind Al ions and so reduce their mobility and uptake. Ohman (1988) emphasized citrates as powerful Al-chelators. The binding of citrate carboxyl groups with the Al-hydrated ion is a basis for reducing their diffusion through the lipid layer of plasmalemma (Akeson and Munns, 1989). Al also forms complexes with fatty acids. It is assumed that chelates of Al with free fatty acids (at concentrations > 200 mM) intensify Al uptake, unlike chelates with esterified fatty

acids. However, the mechanism of activity of this type of tolerance is limited by the fact that chelates are constantly being broken down by microorganisms. Their stable renewal requires a great deal of energy from the plant, which is why this mechanism is less efficient.

Root colonization with mycorrhizal fungi is also an important mechanism of plant adaptation to acid soils with high Al contents and smaller amounts of available phosphorus in warm climatic regions. Ectomycorrhizal fungi bind Al in cell walls, thus reducing its uptake. A crucial role of ectomycorrhizal fungi has been partially proved for individual genotypes cultivated on tropical soils with phosphorus deficits, and in cases where plant root systems were not secreting significant amounts of organic acids (Goedert et al., 1997). Apart from their protective role against Al-induced stress, ectomycorrhizal fungi also increase phosphorus availability to some mycorrhizal plants (e.g. Norwegian spruce).

Internal mechanisms of tolerance

Internal mechanisms of tolerance to Al in Al-tolerant genotypes of cereals become activated after a large amount of Al has penetrated plant cells through the plasma membrane. The activity of this mechanism is based on Al complexation with proteins, organic acids and enzymes, and chelation in cytosols.

An important part in Al detoxication in the plant symplast is played by organic acids that form complexes with Al by accumulating them in specific cell organelles, especially the vacuoles. Al complexation with organic acids, primarily with citric and malic acids, is an important internal mechanism of tolerance to high concentrations of Al. Al detoxication in plant cell cytoplasm is based on Al complexation with proteins, especially with the enzymatic protein calmodulin. Besides calmodulin, other proteins of the metalloprotein group form complexes with Al in cells; they bind Al via the SH group, forming relatively stable complexes. The formed complexes are then transferred into the vacuole by the enzyme tonoplast transferase (Al-ATPase) (Aniol, 1984). The activity of protein kinase in Al-tolerant cultivars has also been found to reduce the toxic levels of Al, compared to sensitive cultivars (Moustakas et al., 1992).

A decrease in cellular Al toxicity may also occur by its complexation with phytochelatins. In small grains, especially in tolerant genotypes, a large number of substances have been found that are able to form chelates with Al.

Genetic mechanisms of tolerance

Plants have genetically controlled mechanisms (present in most phenotypes) and adaptive mechanisms (present in tolerant phenotypes) of overcoming high concentrations of various elements in their nutrient medium.

The environment with all of its factors influence plant organisms, causing high variability in plants of the same inherited background. In addition, genetic sources of tolerance can be very different and scattered in different indivi-

duals of wild populations. Knowing the genetic background of tolerance is greatly important for creating genotypes tolerant to acid medium, and to the presence of high concentrations of Al.

Small grains show different levels of tolerance to acid soils and high contents of Al in soil solution. Barley (*Hordeum vulgare* L.) is the most sensitive of them, followed by wheat (*Triticum aestivum* L.), while oats (*Avena sativa* L.) and rye (*Secale cereale* L.) show higher tolerance. However, differences that have been detected in the tolerance of some genotypes within a species are often greater than those between species.

Genetic characters and gene localization on chromosomes that are responsible for tolerance to acidity and high Al content have been extensively studied in cereals, especially in wheat.

Slootmaker (1974) was one of the first researchers in the world to localize roughly wheat genes for tolerance to soil acidity, specifying the importance of genome D, as well as genomes A and B. Aniol and Gustafson (1984) found the genes responsible for wheat tolerance to Al on the following chromosome arms: 6AL, 7AS, 4BL, 2DL, 3DL, 4DL and 7D, confirming that indeed the genes for Al tolerance exist predominantly in the A and D genomes. In hexaploid wheat, the major genes that affect Al tolerance are localized on the short arm of chromosome 5A and the long arms of chromosomes 2D and 4D (Aniol, 1995).

According to Kerridge and Kronstad (1968), only one dominant gene was responsible for Al tolerance in cross-breeding of the wheat cultivars Duchamp and Brevor, but additional genes were present in the cultivar Atlas 66. This is consistent with findings reported by Campbell and Laffever (1981), who found that Al tolerance to wheat was not merely inherited, but that expression of Al tolerance was additive and highly inheritable. Camargo (1981) showed that the Al tolerance displayed by Atlas 66 was determined by a complex genetic mechanism involving at least two dominant major genes, and possibly some other minor genes. A gene on chromosome 5D had been identified previously, but Berzonsky (1992) found that Al tolerance in Atlas 66 was determined not only by the dominant genes located in genome D, but genomes A and/or B as well. In their studies of different crossbreeding, Rajaram et al. (1991) identified the presence of two complementary dominant genes in one parent, and one recessive gene in two other parents. Other studies have indicated that tolerance to Al is simply an inherited characteristic based on one dominant major gene (Delhaize et al., 1993; Somers and Gustafson, 1995; Somers et al., 1996; Basu et al., 1997). Recently, a connection has been revealed between the restriction fragment length polymorphism (RFLP) marker on chromosome 4DL and a gene for Al tolerance in the wheat cultivar BH 1146 originating from Brazil (Riede and Anderson, 1996). Ramana et al. (2005) determined the genomic structure of the Al tolerance gene (ALMT1) coding for Al-induced malate transporter. The gene was mapped on chromosome 4DL and cosegregates with Al tolerance. Tolerance to Al cosegregates with increased capacity for Al-induced malate efflux. The authors believe that Al tolerance is connected in most wheat genotypes with the function of this gene. Cai et al. (2008) disco-

vered three quantitative trait alleles (QTAs) that are related to Al tolerance. One of them cosegregates with the marker for ALMT1 gene promoter and is situated on chromosome 4DL, while the other two are on chromosomes 3BL and 2A. The two main quantitative trait loci (QTLs) on chromosomes 4DL and 3BL have an additive effect and the SSR markers linked to them can be used in marker-assisted selection aimed to increase wheat tolerance to Al.

Rye is one of the species with the highest tolerance to stress in the family *Triticeae* (Little, 1988; Aniol and Madej, 1996). Its genes for Al tolerance have been localized on chromosomes 3R, 4R, and 6RS (Aniol and Gustafson, 1984). Gallego and Benito (1997) studied gene segregation for Al tolerance and the positions of several isoenzymes in a segregating population and found that Al tolerance in rye was controlled by at least two major dominant and independent loci. The genes coding for isoenzymes Acol and Ndh2 relate to the segregating gene for tolerance to Al on chromosome 6R. Comparing segregation in several rye populations, Hede et al. (2001) discovered that Al tolerance in rye was controlled by several dominant alleles that have different effects on two or three independent loci.

Many triticale cultivars are characterized by high tolerance to Al, but not as high as rye (Hede et al., 2001). Evidently, some wheat genes block the expression of Al tolerance genes from rye, while others enable the expression of rye's tolerance to Al. Aniol and Gustafson (1984) showed that expression of the 6R tolerance to Al depends on which wheat chromosome has been replaced. Gustafson and Ross (1990) found suppressors of rye tolerance to Al on chromosome arms 4AL, 5AL, 6AL, 7BS, 7BL, and 3DS. Similarly, activators of rye tolerance to Al were present on arms 2AL, 5AS, 6BS, 1DS, 1DL, 2DL, 4DL, 5DS, 5DL, 6DL, 7DS, and 7DL.

Barley is the most sensitive of all small grains to the activity of Al. Genetic analyses have shown that barley tolerance to acid soils was inherited by one dominant gene (Stølen and Andersen, 1978) and by multiple alleles (Minella and Sorrells, 1992). Stølen and Andersen (1978) found that tolerance to high soil acidity is regulated by one dominant gene, marked as Pht, on chromosome 4. According to Reid (1971), Al tolerance of the barley cultivars Dayton and Smooth Awn 86 is regulated by one dominant gene, marked as Alp. Echart et al. (2006) reported that barley tolerance to Al depended on one gene and found the RFLP marker Xwg464 on the long arm of chromosome 4H at 21.6 cM distance from the gene.

Examining sweet sorghum (*Sorghum bicolor* L.), Jurandir et al. (2007) identified a gene coding for Al-induced citrate transporter on the locus *Alt_{SB}*, which is believed to be responsible for Al tolerance. It is one of the proteins of the MATE gene family considered to increase Al tolerance by stimulating secretion of citrates from the root.

In rice, 9 QTLs have been identified, including one responsible for root length under non-stress conditions (CRL), three for root length under Al-induced stress (SRL) and five for relative root length (RRL) (Nguyen et al., 2003). Complementary genetic analysis has revealed that QTLs for RRL, mapped on chromosomes 1 and 9, are conserved among different rice populations. Major QTL for RRL, which accounts for 24.9% of the phenotypic variability,

has been found on chromosome 3 of rice that is conserved in all cereals. These results are valuable to breeders, and further studies of these QTLs could make a significant contribution to clarifying the mechanisms of adaptation of different cereals to acid soils.

REFERENCES

- Akeson, M. A., Munns, D. N. (1989): *Lipid bilayer permeation by neutral aluminum citrate and by three alpha-hydroxyl carboxylic acids*. Acta Biochem. Biophys. 984: 200—206.
- Aniol, A. (1995): *Genetics of tolerance to aluminum in wheat (Triticum aestivum L. Thell)*. Plant Soil 123: 223—227.
- Aniol, A., Gustafson, J. P. (1984): *Chromosome location of genes controlling aluminum tolerance in wheat, rye, and triticale*. Can. J. Genet. Cytol. 26: 701—705.
- Aniol, A., Madej, L. (1996): *Genetic variation for aluminum tolerance in rye*. Votr. Pflanz. 35: 201—211.
- Arsenijević-Maksimović, I., Jelić, M., Lomović, S., Kastori, R. (2001): *Genetic and physiological bases of plant tolerance to high concentrations of heavy metals and aluminium*. In: Quarrie, S. A. et al. (eds.) Genetics and Breeding of Small Grains. ARI Serbia, Belgrade, 377—405.
- Basu, U., McDonald, J. L., Archambault, D. J., Good, A. G., Briggs, K. G., Aung, T., Taylor, G. J. (1997): *Genetic and physiological analysis of doubled-haploid, aluminum-resistant lines of wheat provide evidence for the involvement of a 23 kD, root exudate polypeptide in mediating resistance*. Plant Soil 196: 283—288.
- Berzonsky, W. A. (1992): *The genomic inheritance of aluminum tolerance in 'Atlas 66' wheat*. Genome 35: 689—693.
- Blamey, F. P., Edmeades, C. D. C., Wheeler, D. M. (1990): *Role of root cation-exchange capacity in differential aluminum tolerance of Lotus species*. J. of Plant Nutr. 13: 729—744.
- Cai, S., Bai, G. H., Zhang, D. (2008): *Quantitative trait loci for aluminum resistance in Chinese wheat landrace FSW*. Theor. Appl. Genet. 117: 49—56.
- Camargo, C. E. O. (1981): *Wheat improvement. I. The heritability of tolerance to aluminum toxicity*. Bragantia 40: 33—45.
- Campbell, L. G., Lafever, H. N. (1981): *Heritability of aluminum tolerance in wheat*. Cer. Res. Commun. 9: 281—287.
- Carver, B. F., Ownby, J. D. (1995): *Acid soil tolerance in wheat*. Adv. Agron. 54: 117—173.
- Chang, Y. C., Yamamoto, Y., Matsumoto, H. (1999): *Accumulation of aluminum in the cell wall pectin in cultured tobacco (Nicotiana tabacum L.) cells treated with a combination of aluminium and iron*. Plant Cell Environ. 22: 1009—1017.
- Đalović, I., Jocković, Đ., Bekavac, G., Dugalić, G. (2007): *NS hibridi kukuruza za agroekološke uslove Zapadne Srbije*. XII Savetovanje o biotehnologiji. Zbornik radova, Agronomski fakultet Čačak 12—13: 283—294.

- De la Fuente, J. M., Ramirez-Rodriguez, V., Cabrera-Ponce, J. L., Herrera-Estrella, L. (1997): *Aluminum tolerance in transgenic plants by alteration of citrate synthesis*. Science 276: 1566—1568.
- Delhaize, E., Higgins, T. J. V., Randall, P. J. (1991): *Aluminum tolerance in wheat: Analysis of polypeptides in the root apices of tolerant and sensitive genotypes*. In: Plant-Soil Interactions at Low pH. Wright RJ (eds). Kluwer Academic Publishers, Dordrecht, The Netherlands. 1071—1079.
- Echart, C. L., Barbosa-Neto, J. F., Smith Cavalli, S. (2006): *Aluminum tolerance in barley: molecular mapping analyses*. R. Bras. Agrociência, Pelotas, 12: 15—20.
- Eswaran, H., Reich, P., Beinroth, F. (1997): *Global distribution of soils with acidity*. In: Plant-Soil Interactions at Low pH. Moniz AC (eds). Brazilian Soil Sci. Soc. 159—164.
- Foy, C. D. (1984): *Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil*. In: Soil Acidity and Liming. Adams F (ed). American Society of Agronomy, Inc., Madison, WI. 57—97.
- Foy, C. D. (1988): *Plant adaptation to acid, aluminum-toxic soils*. Commun. Soil Sci. Plant Anal. 19: 959—987.
- Foy, C. D., Fleming, A. L. (1982): *Aluminum tolerances of two wheat genotypes related to nitrate reductase activities*. J. Plant Nutr. 5: 1313—1333.
- Gallego, F. G., Benito, C. (1997): *Genetic control of aluminum tolerance in rye (Secale cereale L.)*. Theor. Appl. Genet. 95: 393—399.
- Goedert, W. J., Lobato, E., Lourenco, S. (1997): *Nutrient use efficiency in Brazilian acid soils: Nutrient management and plant efficiency*. In: Plant Soil Interactions at Low pH. Moniz AC (eds). Brazilian Soil Sci. Soc. 97—104.
- Gunse, B., Poschenrieder, Ch., Barceló, J. (1997): *Water transport properties of roots and root cortical cells in proton- and Al-stressed maize varieties*. Plant Physiol. 113: 595—602.
- Gustafson, J. P., Ross, K. (1990): *Control of alien gene expression for aluminum tolerance in wheat*. Genome 33: 9—12.
- Hede, A. R., Skovmand, B., Ribaut, J. M., González-de-León, D., Stølen, O. (2001): *Evaluation of aluminum tolerance in a spring rye collection using two hydroponic screening techniques*. Plant Breeding 31: 49—58.
- Henderson, M., Ownby, J. D. (1991): *The role of root cap mucilage secretion in aluminum tolerance in wheat*. Current Topics in Plant Biochemistry and Physiology 10: 134—141.
- Horst, W. J. (1995): *The role of the apoplast in aluminum toxicity and resistance of higher plants: A review*. Z. Pflanzenernähr. Bodenk. 158: 419—428.
- Jayasundara, H. P. S., Thomson, B. D., Tang, C. (1998): *Responses of cool season grain legumes to soil abiotic stresses*. Advances Agron. 63: 77—151.
- Jelić, M. (1996): *Proučavanje mineralne ishrane pšenice gajene na lesiviranoj smonici*. Doktorska disertacija. Poljoprivredni fakultet Zemun, 1—121.
- Jelić, M., Živanović-Katić, S., Nikolić, O., Milivojević, J., Cvetiković, D., Dugalić, G. (2004): *Perspektive korišćenje genetski tolerantnih biljaka prema aluminijumu za proizvodnju cerealija*. Ekološka istina „Ekoist'04”, Borsko jezero. Zbornik radova, 245—248.

- Johnson, P. A., Bennet, R. J. (1991): *Aluminium tolerance of root cap cells*. J. Plant Physiol. 137: 760—762.
- Jovanović, Z., Djalović, I., Komljenović, I., Kovačević, V., Cvijović, M. (2006): *Influences of liming on vertisol properties and yields of the field crops*. Cereal Res. Commun. 34: 517—520.
- Jurandir, V., Magalhaes, J. V., Liu, J., Guimarães, C. T., Lana, U. G. P., Alves, V. M. C., Wang, Y-H., Schaffert, R. E., Hoekenga, O. A., Piñeros, M. A., Shaff, J. E., Klein, P. E., Carneiro, N. P., Coelho, C. M., Trick, H. N., Kochian, L. V. (2007): *A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum*. Nature Genetics 39: 1156—1161.
- Kerridge, P. C., Kronstad, W. E. (1968): *Evidence of genetic resistance to aluminum toxicity in wheat (Triticum aestivum Vill., Host.)*. Agron. J. 60: 710—711.
- Kochian, L. V. (1995): *Cellular mechanisms of aluminum toxicity and resistance in plants*. Ann. Rev. Plant Physiol. Plant Mol. Biol. 46: 237—260.
- Kovačević, V., Banaj, D., Kovacevic, J., Lalic, A., Jurkovic, Z., Krizmanic, M. (2006): *Influences of liming on maize, sunflower and barley*. Cer. Res. Commun. 34: 553—556.
- Lazof, D. B., Goldsmith, J. G., Rufty, T. W., Linton, R. W. (1994): *Rapid uptake of aluminum into cells of intact soybean root tips*. Plant Physiol. 106: 1107—1114.
- Little, R. (1988): *Plant soil interactions at low pH: Problem Solving — The Genetic Approach*. Commun. Soil Sci. Plant Anal. 19: 1239—1257.
- Marienfeld, S., Schmohl, N., Klein, M., Schroeder, W. H., Kuhn, A. J., Horst, W. J. (2000): *Localisation of aluminum in root tips of Zea mays and Vicia faba*. J. Plant Physiol. 156: 666—671.
- Marschner, H. (1991): *Mechanisms of adaptation of plants to acid soils*. Plant Soil 134: 1—24.
- May, H. M., Nordstrom, D. K. (1991): *Assessing the solubilities and reaction kinetics of aluminous minerals in soil*. In: Soil Acidity. Ulrich B, Sumner M. E. (eds). Springer-Verlag, Berlin 125—148.
- Minella, E., Sorrells, M. E. (1992): *Aluminum tolerance in barley: Genetic relationships among genotypes of diverse origin*. Crop Sci. 32: 593—598.
- Moustakas, M., Yupsanis, T., Symeonidis, L., Karataglis, S. (1992): *Aluminum toxicity effects on durum wheat cultivars*. J. Plant Nutr. 15: 627—638.
- Narro, L. A., Perez, J. C., Pandey, S., Crossa, J., Salazar, F., Arias, M. P. (2001): *Implications of soil-acidity tolerant maize cultivars to increase production in developing countries*. In: N. Ae (eds). Plant nutrient acquisition: New perspectives. NIAES series 4. Springer-Verlag, Japan. 447—463.
- Nguyen, B. D., Brar, D. S., Bui, B. C., Nguyen, T. V., Pham, L. N., Nguyen, H. T. (2003): *Identification and mapping of the QTL for aluminum tolerance introgressed from the new source, Oryza Rufipogon Griff., into indica rice (Oryza sativa L.)*. Theor. Appl. Genet. 106: 583—93.
- Ohman, L. O. (1988): *Equilibrium and structural studies of silicon (IV) and aluminum (III) in aqueous solutions*. 17. Stable and metastable complexes in the system H^+-Al^{3+} citric acid. Inorg. Chem. 27: 2565—2570.

- Pellet, D. M., Papernik, L. A., Jones, D. L., Darrah, P. R., Grunes, D. L., Kochian, L. V. (1997): *Involvement of multiple aluminum exclusion mechanisms in aluminum*. Plant Soil 192: 63—68.
- Puthota, V., Cruz-Ortega, R., Johnson, J. J., Ownby, J. (1991): *An ultrastructural study of the inhibition of mucilage, reaction in the wheat root cap by aluminum*. In: Plant-Soil Interactions at Low pH. Wright, R. J., Baligar, V. C., Murrmann, R. P. (eds.). Kluwer Academic, Dordrecht, The Netherlands 779—787.
- Rajaram, S. M., Kohli, M., Lopez-Cesati, J. (1991): *Breeding for tolerance to aluminum toxicity in wheat*. In: Plant-Soil Interactions at Low pH. Wright, R. J. (eds). Kluwer Academic Publishers, Dordrecht, The Netherlands. 1019—1028.
- Raman, H., Zhang, K., Maron, L., Kochian, L. V., Garvin, D. F., Sasaki, T., Yamamoto, Y., Matsumoto, H., Delhaize, E., Ryan, P. (2005): *Molecular characterization and mapping of almt1, the aluminum-tolerance gene of bread wheat (Triticum aestivum L.)*. Genome 48: 781—791.
- Reid, D. A. (1971): *Genetic control of reaction to aluminum in winter barley*. In: Barley Genetics II. Proc. 2nd. Int. Barley Genet. Symp. Nilan, R. A. (ed). Pullman, Washington State University Press. 409—413.
- Rengel, Z. (1996): *Uptake of aluminum by plant cells*. New Phytol. 134: 389—406.
- Rengel, Z., Reid, R. J. (1997): *Uptake of Al across the plasma membrane of plant cells*. Plant Soil 192: 31—35.
- Riede, C. R., Anderson, J. A. (1996): *Linkage of RFLP markers to an aluminum tolerance gene in wheat*. Crop Sci. 36: 905—909.
- Ryan, P. R., Delhaize, E., Randall, P. J. (1995): *Malate efflux from root apices and tolerance to aluminum are highly correlated in wheat*. Aust. J. Plant Physiol. 22: 531—536.
- Silva, J. R., Smyth, T. J., Moxley, D. F., Carter, T. E., Allen, N. S., Rufty, T. W. (2000): *Aluminum accumulation at nuclei of cells in the root tip. Fluorescence detection using lumogallion and confocal laser scanning microscopy*. Plant Physiol. 123: 543—552.
- Sivaguru, M., Baluska, F., Volkman, D., Felle, H. H., Horst, W. J. (1999): *Impacts of aluminum on the cytoskeleton of the maize root apex. Short-term effects on the distal part of the transition zone*. Plant Physiol. 119: 1073—1082.
- Slootmaker, A. Lj. (1974): *Tolerance to high soil acidity in wheat related species, rye and triticale*. Euphytica 23: 505—513.
- Somers, D. J., Gustafson, J. P. (1995): *The expression of aluminum stress induced polypeptides in a population segregation for aluminum tolerance in wheat (Triticum aestivum L.)*. Genome 38: 1213—1220.
- Somers, D. J., Briggs, K. G., Gustafson, J. P. (1996): *Aluminum stress and protein synthesis in near isogenic lines of Triticum aestivum differing in aluminum tolerance*. Physiol. Plant. 97: 694—700.
- Stevanović, D., Jakovljević, M., Martinović, Lj. (1995): *Rešavanje problema kiselih zemljišta Srbije — preduslov povećanja proizvodnje hrane i zaštite zemljišta. Savetovanje „Popravka kiselih zemljišta Srbije primenom krečnog đubriva „Njival Ca”, Zbornik radova, Paraćin, 7—21*.
- Stølen, O., Andersen, S. (1978): *Inheritance of tolerance to low soil pH in barley*. Hereditas 88: 101—105.

- Sumner, M. E. (2004): *Food production on acid soils in the developing world: problems and solutions*. Proceedings of the 6th International Symposium on Plant-Soil Interactions at Low pH. Matsumoto H. Sendai, Japan. 2—3.
- Takabatake, R., Shimmen, T. (1997): *Inhibition of electrogenesis by aluminum in characean cells*. Plant Cell Physiol. 38: 1264—1271.
- Taylor, G. J. (1988): *The physiology of aluminum phytotoxicity*. In: Metal ions in biological systems: Aluminum and its rule in biology. Sigel, H., Sigel, A. (eds). Marcel Dekker, New York. 123—163.
- Taylor, G. J. (1991): *Current views of the aluminum stress response: the physiological basis of tolerance*. Current Topics Plant Biochem. Physiol. 10: 57—93.
- Taylor, G. J. (1995): *Overcoming barriers to understanding the cellular basis of aluminum resistance*. Plant Soil 171: 89—103.
- Taylor, G. J., McDonald-Stephens, J. L., Hunter, D. B., Bertsch, P. M., Elmore, D., Rengel, Z., Reid, R. J. (2000): *Direct measurement of aluminum uptake and distribution in single cells of Chara corallina*. Plant Physiol. 123: 987—996.
- Van Wambeke, A. (1976): *Formation, distribution and consequences of acid soils in agricultural development*. In: Proceedings of Workshop on Plant Adaptation to Mineral Stress in Problem Soils. Wright, M. J., Ferrari, S. A. (eds). Spec. Publ. Cornell Univ. Agric. Exp. Stn., Ithaca, NY. 15—24.
- Von Uexkull, H. R., Mutert, E. (1995): *Global extent, development and economic impact of acid soils*. Plant Soil 171: 1—15.
- Wagatsuma, T. (1983): *Characterization of adsorption sites for aluminum in the roots*. Soil Sci. Plant Nutr. 29: 499—515.
- Wagatsuma, T., Yamasaku, K. (1985): *Relationship between differential aluminum tolerance and plant-induced pH change of medium among barley cultivars*. Soil Sci. Plant Nutr. 31: 521—535.
- Welcker, C., The, C., Andreau, B., De Leon, C., Parentoni, S. N., Bernal, J., Felicite, J., Zonkeng, C., Salazar, F., Narro, L., Charcosset, A., Horst, W. J. (2005): *Heterosis and combining ability for maize adaptation to tropical acid soils: implications for future breeding strategies*. Crop. Sci. 45: 2405—2413.
- Zhang, G., Taylor, G. J. (1991): *Effects of biological inhibitors on the kinetics of aluminum uptake by excised roots and purified cell wall material of aluminum tolerant and aluminum-sensitive cultivars of Triticum aestivum L.* J. Plant Physiol. 138: 533—539.

МЕХАНИЗМИ АДАПТАЦИЈЕ СТРНИХ ЖИТА НА КИСЕЛОСТ ЗЕМЉИШТА

Ивица Г. Ђаловић¹, Ивана В. Максимовић²,
Рудолф Р. Кастори², Миодраг Ж. Јелић³

¹ Институт за ратарство и повртарство, Нови Сад,
Максима Горког 30, 21000 Нови Сад, Србија

² Пољопривредни факултет, Нови Сад, Трг. Д. Обрадовића 8,
21 000 Нови Сад, Србија

³ Пољопривредни факултет, Зубин Поток, Ј. Анжујске бб,
38228, Зубин Поток, Србија

Резиме

Кисела земљишта ограничавају биљну производњу на 30—40% укупних, као и до 70% потенцијално обрадивих светских површина. Кисела реакција ових земљишта и низак садржај најважнијих биљних хранива, пре свега Р и Са су ограничавајући фактори постизања високих и стабилних приноса гајених биљака. Поред киселе реакције, ова земљишта карактерише веома често и повећан садржај токсичних облика Al, Fe и Mn, као и недостатак или смањена приступачност Р, Са, Mg и неких микроелемената, посебно Мо, Zn и В. Токсичност Al се сматра најважнијим фактором који ограничава раст биљака на киселим земљиштима. Механизми адаптације стрних жита на кисела земљишта могу се поделити на: спољашње, унутрашње (физиолошке) и генетичке. Дејство спољашњих механизма толерантности заснива се на имобилизацији Al у ћелијском зиду, истисању Al кроз плазма мембрану, успостављању рН баријере у ризосфери и лучењу органских киселина, фосфата, хелата и других лиганата кореном у спољашњу средину. Унутрашњи механизми толерантности заснивају се на комплексирању Al са протеинима, органским киселинама и ензимима, као и хелатизирању у цитоплазми. Стрна жита испољавају различиту толерантност према киселости земљишта и повећаном садржају Al у земљишном раствору. Најосетљивији је јечам, затим пшеница, док већу толерантност испољавају овас, тритикале и раж.

*Vladan D. Mirjanić¹, Radmila R. Arbutina¹,
Jovan P. Šetrajčić², Ljubiša D. Džambas³*

¹ Faculty of Medicine, Department of Stomatology, University of Banja Luka,
Save Mrkalja 14, Banja Luka, Republic of Srpska, BiH

² Department of Physics, Faculty of Sciences, University of Novi Sad,
Trg D. Obradovića 4, Novi Sad, Serbia

³ Faculty of Medicine, Department of Stomatology, University of Novi Sad,
Hajduk Veljkova 3, Novi Sad, Serbia

PHYSICAL PROPERTIES OF THIN FILMS ON IMPLANT-MATERIALS

ABSTRACT: In this paper, we present the analysis of thin films in implant materials. Based on preliminary results, it can be concluded that thin polymer films between bone and implant achieve biocompatibility. In addition, analysis will be conducted for other characteristics, such as bioinertia, and biofunctionality.

KEY WORDS: Thin Films, Implant Materials, Bio-compatibility, Physical properties

INTRODUCTION

The development of civilization has followed the development of new implantology ideas and new materials which have been used for that purpose and a large number of discovered archaeological finds can testify to that, as well as an immense number of written documents.

During the 1970', with the appearance of Brånemark and his implants, a new era of implantology began. This period is characterized by the appearance of new, as well as the modification of the old designs, and the occurrence of new surgical techniques. The aim of modern implantology is to establish the normal function of mastication, speech, aesthetic appearance, health and comfort, regardless of the type of dental system injury, where the implant will have a central place in all of these rehabilitations. The base of this new philosophy is osteo-integration and a range of prerequisite conditions, so it could be fully realized. Osteo-integration or biointegrity of implants is founded on the hypothesis that, after implantation, a process of remodulation of the bone and osteo-genesis, the implant will integrate into the bone tissue and take over the functional load of prosthetic substitute.

By establishing this term, Bronemark is introducing new principles or factors that can influence attaining this morphological and functional relation between the bone and surface of the dental implant.

Adoption of these principles in everyday work includes implicitly expertness in basic principles of the central injury, inflammation, bone cicatrisation, successive addition and remodeling of the bone, as well as physical characteristics of the implant and its surface. Implantation materials regardless of type and nature of the implantation, in the first place should be biologically acceptable.

Closer characteristics that implantation materials should have are:

- That it is compatible with tissues which it is being implanted with
- That it is not toxic to the tissue and human body
- That it does not cause immunological reactions
- That it is not potentially carcinogenic
- That it is resistant to the corrosive influence of the living environment
- That it has acceptable physical and mechanical properties
- That it can be manufactured in to acceptable geometrical shapes
- That it can be sterilized and
- That they must be good thermic and electric insulators

All the above-mentioned conditions are incorporated into the following terms: biocompatible, bioinert, and biofunctional. These properties fill up the ultra thin coating (ultra thin films or nanofilms) as it is expected that ultra thin coatings represent a biophysical system with few free electrons and then the mechanic oscillations of atoms must be smothered.

Biomaterials and biomechanical properties are of fundamental importance for the success or failure of dental implants. Biomaterials are „biological substituents” from which „biological spare parts” can be produced. Biomaterials must not act as an antigen but should successfully induce osteogenesis. Over the past sixty years, research has been carried out on the biomaterials and biomechanical design for surgical implants. The result of this research was the discovery that a wide spectrum of materials can replace living tissues, and among those dental implants take one of the more important places on a scale.

In proportion to biochemical electro-chemical interaction of vital tissue and non-vital material, all biomaterials, according to Osborne (Osborne, 1985) or according to the level of biocompatibility, can be divided to:

- Biotolerant materials (Co, Cr, Mo-alloys, Fe, Cr, Ni-alloys, PMMA)
- Bioinert materials (titan CP, tantalum, bioceramics)
- Bioactive materials (hydroxapatite, Ca-phosphates)

Regarding their origin, biomaterials are divided into autolog, allogeneic, heterolog and alloplastic.

In most cases, dental implants are classified by their division to metal and non-metal. In essence, they are alloplastic materials.

The following come under classification of metals and alloys: titanium CP (Ti), titanium-aluminum-vanadium (Ti-6Al-4V), cobalt-chromium-molybdenum (Co-Cr-Mo), tantalum (Ta), ferrous-chromium-nickel (Fe-Cr-Ni) 316L, and non-metals are classified as: ceramics, hydroxapatite (Ha), carbon, polymers (PMMA).

METALS FOR IMPLANTS — TITANIUM

For its electro-chemical properties, titanium is considered as the most compatible metal, and is often used in modern stomatology, implantology and prosthetics (Fraker, 1988; Lenz, 1998).

Martinović and his associates have researched the corrosion resistance of titanium alloys, used for orthopedic implants, in body liquids. Spectrum-electro-chemical research on titanium alloys *in vitro* has confirmed the creation of protective oxidation film on the alloy surface. Comparing the stability of titanium in different corrosive baths with results on other alloys, the authors conclude that titan is the most stable. Although titan is corrosive stable, combined with various metals it can create strong galvanic segments, therefore it is necessary to be cautious while combining titan with other alloys. Some authors consider that precious metals (Au, Ag, Pd) combined with titanium make very small galvanic currents. Combined with the Ni-Cr-Be alloys those currents are more considerable so such combination should be avoided. There has also been research on the influence of amino acids on the corrosiveness of titanium alloys and it is concluded that the existence of tryptophan in the alloy does not affect the corrosive stability of titanium, unlike cysteine, which increases it.

Titanium is a non-precious, extremely reactive material. For that reason, after the titanium is processed, oxide (TiO_2) is formed on its surface (Stamenković, 2003). The thickness of the oxide layer is 15—50 μm . This oxide is resistant to corrosion and is bioinert. Biocompatibility is affected by the high dielectric constant of titanium oxide too. Consequently, biocompatibility derives from titan oxide, and not from the pure metal. Mechanical properties of titanium satisfy all criteria which metal should possess to be used in implantology. It has extremely high tightening strength, which enables it to endure great loads. In addition, it has the property of being forged that makes it resistant to impact. Under a strong force, it plastically deforms but it does not break. On the titanium surface, bone formation is implantopetal. That means the bone is being formed from the bony-bed of implant towards the implant. Firstly the connective tissue callus is formed, then osteoid tissue and on the end lamellar bone. Electronic — microscopic researches show that there is a zone (20—40 μm) in which there is noncalcified coat of base substance and collagen filaments belonging to the zone. It means that there is no direct contact between the implant and alveolar bone.

Coating titanium with plasma has the aim of increasing the titan and alveolar bone contact surface. It is obtained by putting particles of melted metal by means of a plasma spout. The thickness of the plasma coat is 30—40 μm . On the surface of implant, there are pores that are interlinked. By the process of spouting plasma, the implant surface is increased by 5 to 10 times. That way the contact surface of the implant and bone tissue is increased too. Presence of pores also stimulates formation of bone tissue and has positive effect on implant integration.

The most recent research show that parts of metal in plasma tissue could be discovered in pre-implant bone tissue. The appearance of such particles is followed by signs of metallosis. To overcome this problem it is necessary to

make an additional enlargement of the implant surface. That can be achieved by sanding, acid erosion, special laser techniques and, in the more recent times, by enlargement of titanium-oxide coat.

NONMETALS FOR IMPLANTS — CERAMIC MATERIALS

A number of ceramic materials are used in implantology and nowadays mostly calcium-phosphate materials in form of hydroxapatite (H, A) and tricalcium phosphate (T, C, P). These materials stimulate the direct apposition of bone tissue on its surface. This means that the process of bone formation is a combination of implantopetal and implantofugal methods of bone forming.

Electronic-microscopic researches show that in the area of these materials there is a direct connection with bone tissue. There is no layer of connecting tissue like in case of pure titan. Titan implants, which have their surface coated with calcium phosphate ceramics, should combine mechanical characteristics of metal and osteo filling abilities of ceramics. Research results show that implants with calcium phosphate show much greater apposition of bone. In clinical practice calciumphosphate ceramic (CFK) materials are used, and coating techniques differ too.

For applying CFK to a metal surface a plasma „spray” technique is mostly used. Synthetic hydroxapatite is warmed up to 1200—1500°C. The way this is done is with an electric arch in a presence of argon gas. This technique is similar to that used for coating plasma over titan. HA particles of 40 μm are coated over the previously prepared metal surface. The metal surface is uneven and porous, as it has been previously treated by sanding and acid erosion. HA particles penetrate into the pores and uneven the surface of metal; after cooling the mechanical relation of HA and metal surface is established. Although the perfected technique of coating metal with CFK, research has shown, the possibility that partial absorption of material can occur because reabsorbed particles have been discovered in pre-implant tissue.

ULTRA THIN COATINGS

At this stage we shall propose the nanocoating model and analyze their thermic and electric properties. The nanocoat model is shown in Fig. 1.

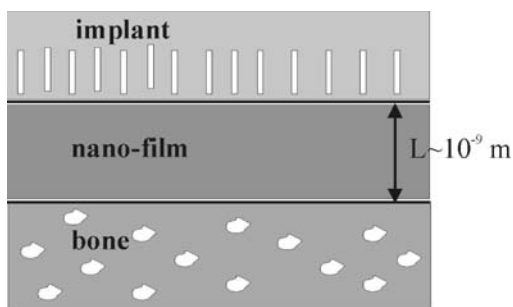


Fig. 1 — Model of nanofilm layer

The nano coat should prevent the direct contact between implant and bone for the above mentioned requests, particularly in the sphere of chemical — physical properties. It is known that (T o š i ć, 1987) structures with markedly diminished dimension can have considerably changed properties regarding the relation to spatially limited structures (balk). That way we can see that electronic nanofilms have a considerably better thermal and electrical insulation properties comparing to corresponding balk structures.

Theoretical analysis of an electronic system in very (extremely) limited and structures with disturbed translational symmetry has found the spectrum of electrons and inner energy (Š e t r a j č i ć, 1990; Š e t r a j č i ć, 2007). Thermodynamic analysis is specified by the electronic portion (share) in the specific heat of ultra thin film coatings. That is shown in Fig. 2. As we can see, the thinner the coating, the more expressive the insulating properties of the coating are, or specific heat is lower.

Analog analysis has shown the phonon portion in specific heat as in Fig. 3, where we can see that the insulating properties are even more pronounced than in the electronic share.

Complete and complex analysis of the transportation properties of nano-coatings from a physics point of view can be acquired only if the system of electrons and phonons, in extremely limited spatial and structures with disturbed translational symmetry are considered.

This means that the electronic and phonon influences (T o š i ć, 2006) on the insulating properties must be added up. Dependence of electro-phonon share in specific heat is shown in Fig 4.

Ultra thin coatings have all of the good insulating properties and can satisfy all of the required thermal and electric coating properties needed in implantology.

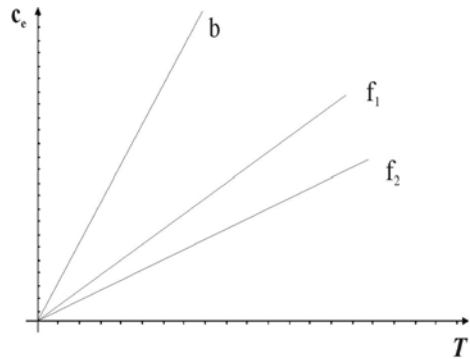


Fig. 2 — Electron specific heat in nanofilms

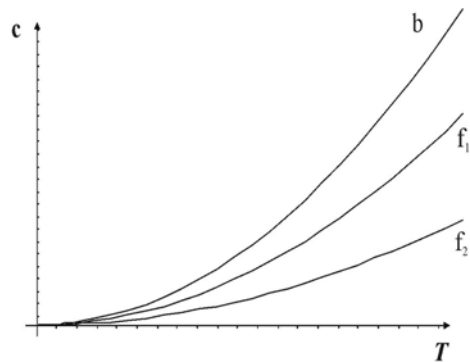


Fig. 3 — Phonon specific heat in nanofilms

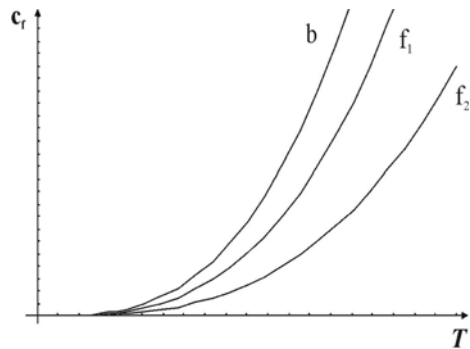


Fig. 4 — Total specific heat in nanofilms

CONCLUSIONS

Here we have analyzed biomaterials and their biomechanical properties in dental implantology with special retrospection to coatings — thin films that must be ultra thin, or nanofilms. Considering their good insulating properties, it is necessary to change drastically the technology of their coating. Only in that way the characteristics of material used in implantology can be satisfactory.

REFERENCES

- Brånemark, P., et al. (1969): *Scand. J. Plast. Reconstr. Surg.* 3:81.
Osborn, F. J. (1985): *Implantat Werkstoff Hydroxylapatit Ceramic*, Quintessence, Berlin.
Fraker, A. C. (1988): *Corrosion of metallic implants and prosthetic devices*, in: *Specific industries and environments*, National bureau of standards, New York.
Lenz E. (1998): *Titan als Werkstoff für Kombiniert Festsitzende und Abnehmbaren Zahnersatz*, Barlingen, Spittaverlag.
Stamenković, D., et al. (2003): *Dentistry Materials*, Faculty of Dentistry, Belgrade 2003. (in Serbian)
Tošić, B. S., et al. (1987): *Phonons In Broken-Symmetry Structures*, Phys. Rev. B 36:9094.
Šetrajčić, J. P., et al. (1990): *Phonon Spectra In Superconducting Ceramics*, Physica Scripta 42:732.
Šetrajčić, J. P., et al (2007): *Phonon Thermodynamics In Crystalline Nanofilms*, Mat. Sci. Forum 555:291.
Tošić, B. S., et al. (2006): *Mechanical Oscillations And Charge Carriers In Nanostructures*, Mat. Sci. Forum 518:47.

ФИЗИЧКЕ ОСОБИНЕ ТАНКИХ ФИЛМОВА НА ИМПЛАНТ-МАТЕРИЈАЛИМА

Владан Д. Мирјанић¹, Радмила Р. Арбутина¹, Јован П. Шетрајчић²,
Љубиша Д. Џамбас³

¹ Медицински факултет, Департман за Стоматологију, Универзитет у Бањој Луци, Саве Мркаља 14, Бања Лука, Република Српска, БиХ

² Департман за физику, Природно-математички факултет, Универзитет у Новом Саду, Трг Д. Обрадовића 4, Нови Сад, Србија

³ Медицински факултет, Департман за Стоматологију, Универзитет у Новом Саду, Хајдук Вељкова 3, Нови Сад, Србија

Резиме

У овом раду смо презентовали анализу танких филмова на имплантним материјалима. На основу прелиминарних резултата, може се закључити да танки полимерни филмови између кости и импланта задовољавају био-компатибилност. Ове анализе су корисне и за друге карактеристике, као што су био-инерција и био-функционалност.

*Goran S. Marković¹, Miroslav A. Ćirković²,
Stevan A. Maletić², Nikolina J. Milošević²*

¹ Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32000 Čačak, Serbia

² Faculty of Agriculture, University of Novi Sad, Trg D. Obradovića 8, 21000 Novi Sad, Serbia

A CONTRIBUTION TO THE DATA ON TENCH (*TINCA TINCA* L., CYPRINIDAE, PISCES) DISTRIBUTION IN SERBIA

ABSTRACT: The studies on the ichthyofauna of Serbia that have been conducted so far indicate the sporadic tench (*Tinca tinca* L.) presence in typical habitats. The hydroenvironmental conditions necessary for the survival of this fish species are highly variable and, generally, are more favourable in the northern part of the country (the Vojvodina Province). Although the tench presence has been registered at over 30 sites, there is a tendency of a decrease in population abundance. This is due to the reduction of the surface area of the macrophyte-covered flood zones induced by the damming and regulation of rivers, high water level fluctuations, and water amount reduction in the majority of watercourses, a massive increase in the number of allochthonous phytofagous species, reservoir sediment deposition, water quality deterioration and other factors. The tench conservation status in Serbia is officially designated as Low Risk — Least Concern. It may be transferred to a higher category if the tendency towards deterioration of survival conditions for the species should continue.

KEY WORDS: distribution, endangerment, protection, Serbia, tench

INTRODUCTION

Anthropogenic activities have dramatically sped up the process of succession of species which is induced by the evolutionary changes in a number of existing biocenoses. The aquatic environment and its hydrobionts, particularly the fish, have been subjected to severe changes. Apart from the global effect being primarily manifested through the deterioration of environmental survival conditions, the ichthyofauna of certain regions has been substantially altered by the introduction and translocation of certain species that are frequent causes of disturbances in the established equilibria. The ichthyofauna of Serbia has also experienced dramatic changes over the last decades.

The Republic of Serbia (its surface area being 88 361 km²) is situated in the central part of the Balkan Peninsula. A part of the territory located north

of the Danube (2783 km long, 588 km in Serbia) and the Sava (945 km, 206 km in Serbia) rivers, the Vojvodina Province (21 506 km²), is characterised by lowlands and a wide range of slow-flowing and stagnant water bodies. The highlands dominating Central Serbia (3/4 of the state territory) are distinguished by the wealth of torrential rivers, rivulets, springs, and upland reservoirs. Larger rivers in this part of Serbia include the Zapadna Morava (308 km), the Južna Morava (295 km), the Ibar (272 km) and the Drina (346 km, 220 km in Serbia). A number of lakes (the most famous of them being Palić and Ludoš) and 85 reservoirs, the Đerdap-dam I (surface area 253 km²) being the largest, contribute to the wealth of water in Serbia. Most river courses in Serbia flow directly or indirectly (from more than 97% of the territory) into the Danube (the Black Sea Basin), the waters from less than 3% of the state territory flowing into the Aegean and Adriatic Seas (Anonymous 2003).

The overall deterioration tendency of the water quality of most water courses in Serbia has been observed in Serbia over recent decades, as reflected by eutrophication and chemical, radioactive and other types of pollution (Ostojić et al., 2005; Bogdanović, 2006; Marković and Lenhardt, 2007).

The high variability of environmental conditions has induced high diversity of ichthyofauna, including a permanent or occasional presence of 94 species from 26 families and a pronounced predominance of the representatives of the Cyprinidae family (50 species) (Simonović and Nikolić, 1997; Karadžić and Mijović, 2007). The diversity of the ichthyofauna has been increased by a large number of non-native species — a total of 22 from 10 families (Lenhardt et al., 2006; Simonović, 2006). The introduction of these species into the water courses of Serbia started in the 1930s and it was intensified in the second half of the 20th century (Holčík, 1991), exhibiting a tendency of the continuous expansion (Maletín et al., 1997; Cakić et al., 2004; Simonović et al., 2006).

The tench (*Tinca tinca* L.) is a cyprinid species of Euro-Siberian origin widely distributed in the watercourses throughout Europe, including the Balkan Peninsula (Wheeler, 1978; Simonović, 2006). Although globally studied, the tench has not been fully explored in Serbia. Although the first record of the species in the ichthyofauna of Serbia was made in the 1860s (Pančić, 1860), later publications, mostly handbooks, provide only general information on the morphology, ecology and breeding of this species (Vuković and Ivanović, 1971; Bojčić et al., 1982; Hristić and Bunjevac, 1991; Ćirković et al., 2002). More intensified research on the ichthyofauna of Serbia's watercourses conducted in 1990s revealed the presence of tench in the Danube and tributary rivers (Hegediš et al., 1992; Janković et al., 1994; Marković and Simović, 1994; Maletín et al., 1996). The results of the above analyses suggest a low percentage of the species (generally accounting for less than 1% of the individual abundance and biomass of local ichthyofauna).

MATERIALS AND METHODS

The little information available on the ecology of the species has motivated the authors to engage in this study. Some data on tench distribution have been provided by the Fishery Improvement Programmes for certain regions in Serbia. The study has been particularly focused on registering tench habitats larger than 0.1 km² in still and slow-flowing waters. These biotopes are typical for this species (Wright and Giles, 1991; Gonzales et al., 2000). The study is a short review of both the current state of tench populations in certain regions of Serbia and the degree of habitat endangerment.

RESULTS AND DISCUSSION

Tench in Serbian open waters

The presence of tench has been registered at more than 30 sites throughout Serbia (Fig. 1, Tab. 1), the majority of them being in the Vojvodina Province (1—21), a part of the Pannonian Basin located in Serbia. The dense hydrographic network of this part of the country includes various types of water bodies (rivers, rivulets, flood zones, pools and bogs, canals, lakes and reservoirs).

Tab. 1 — Main characteristics of tench (*Tinca tinca*) habitats in Serbia

Item	Ecosystem	Area	Biotope	Main purpose of use
(1)	Palić Lake	5.6 km ²	natural	recreation, tourism
(2)	Ludoš Lake	3.6 km ²	natural	irrigation, protected area
(3)	Čik Canal	95 km long	artificial	irrigation, part of DTD Canal
(4)(5)	Vrbas—Bezdan Canal	90 km long	artificial	irrigation, part of DTD Canal
(6)	Odžaci—Sombor Canal	13 km long	artificial	irrigation, part of DTD Canal
(7)(8)	Kosančić—Mali Stapar Canal	21 km long	artificial	irrigation, part of DTD Canal
(9)	Bački Petrovac—Karavukovo Canal	52 km long	artificial	irrigation, part of DTD Canal
(10)(11)	Banatska Palanka—Novi Bečej Canal	147 km	artificial	irrigation, part of DTD Canal
(12)	Kikinda Canal	50 km	artificial	irrigation, part of DTD Canal
(13)	Jegrička River—Canal	65 km	semi-natural	irrigation, angling
(14)	Zobnatica Reservoir	2.5 km ²	artificial	Irrigation, recreation
(15)	Mt. Fruška Gora Reservoirs	≈ 1.2 km ²	artificial	irrigation, recreation
(16)	Borkovac Reservoir	0.7 km ²	artificial	irrigation, recreational fishing
(17)	Bela Crkva Lakes	1 km ²	artificial	recreation, angling
(18)	Carska Bara Bog	11 km ²	natural	special nature reservation
(19)	Kovilj — Petrovaradin Marshes	5.5 km ²	natural	special nature reservation
(20)	Obedska Bara Bog	7 km ²	natural	special nature reservation
(21)	Pančevački Rit Swamp	400 km ²	natural	irrigation

(22)	Zasavica Bog	11.5 km ²	natural	special nature reservation
(23)	Sava Lake	0.8 km ²	artificial	water supply, recreation
(24)	Silver Lake	4 km ²	artificial	recreation, angling
(25)	Đerdap-dam I Reservoir	253 km ²	artificial	hydroelectric production
(26)	Đerdap-dam II Reservoir	92 km ²	artificial	hydroelectric production
(27)	Perućac Reservoir	12.4 km ²	artificial	hydroelectric production
(28)	Međuvršje Reservoir	1.5 km ²	artificial	hydroelectric production
(29)	Ćelije Reservoir	3.4 km ²	artificial	water supply
(30)	Batlava Reservoir	3.3 km ²	artificial	water supply
(31)	Brestovac Reservoir	0.8 km ²	artificial	water supply
(32)	Krupac Lake	0.4 km ²	natural	water supply
(33)	Vlasina Reservoir	16 km ²	semi-natural	hydroelectric production

Record of the presence of tench in the northernmost parts of Serbia was made in Palić (1) and Ludoš lakes (2) of Aeolian origin. The lakes are located between the Danube and Tisa rivers next to the state border with Hungary (Fig. 1). Lake Palić is an important tourist destination in this part of Europe. The depth of 3—4 m, mean water t° of about 10°C, the muddy bottom, and the dense macrophyte vegetation cover make the above ecosystem a favourable habitat for this species. Until the 1960s, the ichthyofauna of the lake was characterised by markedly excessive production of carp (*Cyprinus carpio*) — making up 98% of the total ichthyofauna biomass. However, the lake experienced environmental catastrophe towards the end of the 1960s. Drastic deterioration of the lake water quality induced by permanent waste water discharges from the city of Subotica (8 km away) and other populated areas as well as from agricultural sources led to large-scale death of fish (the essential reason being high silt concentrations of H₂S) in 1971 (Maletić et al., 1998a). Over the period 1971—1976, lake restoration (emptying of the lake, silt removal and re-filling) and stocking were performed. This resulted in the establishment of a dynamic equilibrium. Apart from the Asian herbivore, primarily the grass carp (*Ctenopharyngodon idella*), in the period 1976—1982, the tench was also introduced at the rate of 60—100 kg/year. This induced a moderate increase in the species abundance followed by a decrease in its number as early as in the 1990s due to the deterioration of ambient conditions (Đukić et al., 1991). Currently, the lake ecosystem is characterised by low numbers of the tench (accounting for about 1% of the total fish catch). Lake Ludoš is located in an urban area 12 km away from the town of Subotica. It is supplied with water through the Ludoš-Lake canal that receives purified water from Lake Palić and the Kereš Brook. It is surrounded by farms that use its water for irrigation. This shallow lake (with an average depth of 1m) and its surroundings marked by diverse habitats — a swamp, a meadow and a steppe (the total area of 5.93 km²), were included in the Ramsar List of Wetlands of International Importance in 1977 (Ramsar Sites Database, 2008). Notwithstanding protection measures, the intensified eutrophication of the ecosystem (Bogdanović, 2006) and a decrease in the number of fish, primarily of the *C. carpio* and the tench, have been observed in recent years.

Most tench records (3—13) have been made in the Danube-Tisa-Danube (DTD) Hydrosystem canal network. It is a unique system of canals and dams intended for the irrigation and quality improvement of arable areas, the impro-

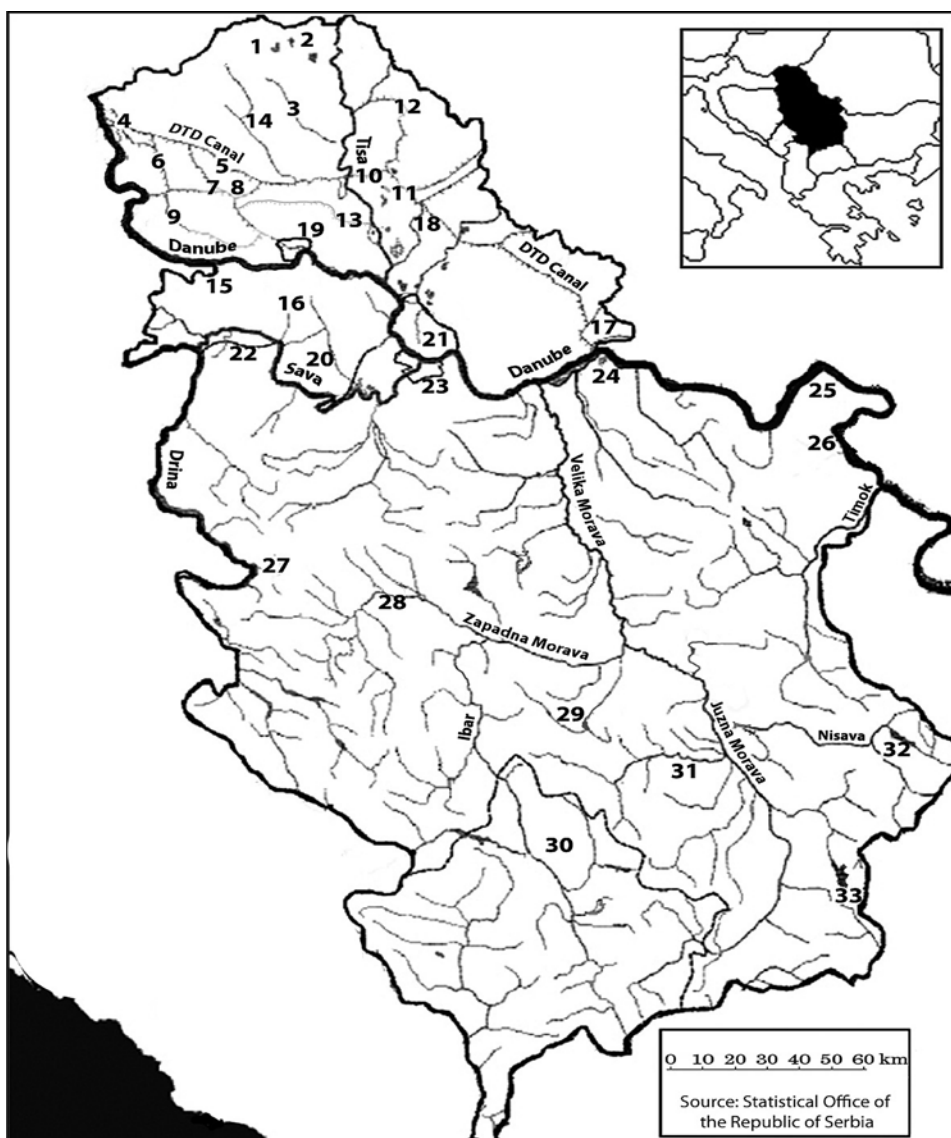


Fig. 1 — Current distribution of tench (*Tinca tinca*) in Serbia

- 1) Palić Lake, 2) Ludoš Lake, 3) Svetičevo, 4) Šebeš Fok 5) Crvenka, 6) Odžaci, 7) Ruski Krstur, 8) Kruščić, 9) Karavukovo, 10) Novi Bečej, 11) Melenci, 12) Novo Miloševo, 13) Temerin, 14) Zobnatica Reservoir, 15) Fruška Gora Reservoirs, 16) Borkovac Reservoir, 17) Bela Crkva Lakes, 18) Carska Bara Bog, 19) Kovilj—Petrovaradin Marshes, 20) Obedska Bara Bog, 21) Pančevački Rit Swamp, 22) Zasavica Bog, 23) Sava Lake, 24) Silver Lake, 25) Đerdap-dam I Reservoir, 26) Đerdap-dam II Reservoir, 27) Perućac Reservoir, 28) Međuvršje Reservoir, 29) Čelije Reservoir, 30) Batlava Reservoir, 31) Brestovac Reservoir, 32) Krupac Lake, 33) Vlasina Reservoir

vement of water supply, the evacuation of waste waters and the development of fisheries (Štanko vić, 2000). The main canal is 248 km long, the whole system encompassing 700 km of secondary canals and about 10 000 km of accessory channels. The total surface area of the soil covered by the Hydrosystem is 12 700 km². The DTD system construction in the 1947—1997 period resulted in a drastic reduction of the flood zones. Despite the drainage of large wetland areas, ambient conditions have been preserved in certain parts of the canal network enabling tench survival.

The profiles (3), (5), (6), (9), (10), (11) and (12) showed variations in the individual (0.23—4.2%) and biomass (0.05%—2.5%) presence of the tench in the total ichthyofauna catch (Malet in et al., 2005; Malet in et al., 2006). Higher abundance of the tench has been registered in the Bezdan profile near Šebeš Fok (4) — 7% of individual fish and 13% of the biomass, in the Ruski Krstur (7) — 15% and 20% and in the Kruščić profile (8) — 18% and 24%, respectively (Malet in et al., 2007). The high tench abundance at these sites is due to a low degree of devastation of habitats and rich macrophyte vegetation providing the species with favourable survival conditions. The profiles (7) and (8) are located in the Kosančić — Mali Stapar Canal ecosystem where the first record of female largemouth bass (*Micropterus salmoides*) in the watercourses of Serbia has been made (Malet in et al., 1997). The massive increase in largemouth bass has not affected the total tench population. However, the introduction of grass carp (*Ctenopharyngodon idella*) aimed at preventing the expansion of macrophyte vegetation (Malet in et al., 2007a) can cause disturbance of the overall environmental conditions in the above habitats and tench endangerment.

As regards the ichthyofauna of the Temerin profile (13) of the Jegrička River (Canal), the individual and biomass presence of the tench was 2.69% and 1.01%, respectively. The Jegrička is the longest artificial watercourse in Vojvodina turned by hydromelioration into a canal and linked within the DTD Hydrosystem. The last 15 km of the watercourse have been converted into a fish pond, stocked with quality fish fry and protected as a Nature Park. This habitat meets the requirements essential for the massive increase in the presence of limnophilous fish species such as tench (Šip oš et al., 2007).

Individual tench records have been made in a number of reservoirs in Vojvodina including Zobnatica on the Krivaja River (14) and those on the southern slopes of Mt. Fruška Gora (15) — Moharač (67 ha), Šot (33 ha) and Bruja (15 ha). Frequent recreational fishing destinations in Vojvodina also include the Borkovac Reservoir (16) made by the damming of the Borkovac Brook in 1971. Continuous stocking in the 1980s led to increases in the carp and tench populations (record was made of individual fish mass exceeding 1.2 kg). Recreational fishermen are also attracted to the reservoirs located within and surrounding Bela Crkva, a town near the Romanian border (17). The above lakes (7 in all) were formed by being filled with ground water following gravel exploitation. Potential reasons for the decrease in tench numbers in the microreservoirs include excessive catch and *C. idella* introduction.

The reduction in the tench distribution area in Vojvodina resulted primarily from river regulations and wetland drainage. Hence the high importance of

the sites (18), (19) and (20) that are considered as refuge for this and other endangered species.

Carska Bara (18) is one of the largest Serbian bogs, located 17 km southwest of the town of Zrenjanin at the confluence of the Begej River into the Tisa. Its superb biodiversity (particularly of the ornithofauna composed of 240 registered species) was the reason for the inclusion of the region, the surface area thereof being 17.67 km², in the Ramsar List (Ramsar Sites Database, 2008) in 1996. Notwithstanding the overall favourable environmental conditions, individual tench presence in the fish fund has been extremely low (0.37—0.74% of the total ichthyofauna comprising 24 species) (Kostić and Maletić, 1992). Recent studies do not report any increases in the total population of the species. Possible reasons include considerable fluctuations in the water level induced by low precipitation and global warming as well as the massive presence of the bird colonies of herons (*Ardea* sp.) and cormorants (*Phalacrocorax* sp.), major fish predators.

A low individual tench presence is also characteristic of Kovilj-Petrovaradin Marshes (19), complex wetlands and forests, located in the inundation area of the middle course of the Danube River, near the City of Novi Sad. The region (its surface area being 48.4 km²) has been proclaimed Special Nature Reserve by the Decree of the Government of the Republic of Serbia (The Official Gazette of the Republic of Serbia, 27/98) due to the biota diversity enhanced by the 46 fish species making up the ichthyofauna of the region. Intensive traffic on the Novi Sad — Belgrade highway passing through the above region is a major factor endangering the autochthonous habitat.

The flood zone of the Sava River, 40 km west of Belgrade, is the site of the Obedska Bara (20), a large swampy forest area stretching along the Sava River (a truncated meander of the Sava). Initial protection measures for this region were taken as early as in 1874. This authentic complex of diverse aquatic and terrestrial biocenoses (the surface area being 175 km²) was included in the Ramsar List (in 1977). Irrespective of the protection measures, constant expansion of surrounding agricultural areas and water level fluctuations have caused a drastic reduction in the abundance of tench which accounted for 7% of the total catch of ichthyofauna in the 1980s (Budačević et al., 1983).

The high environmental adaptability of tench to deteriorated hydrochemical survival conditions has been confirmed by the fish presence recorded in the Pančevački Rit Swamp (21). Its large wetlands (having a surface area of 399.9 km²) located on the right bank of the Danube in a broader area of the city of Belgrade had been incessantly flooded until World War II. Thereafter, they were partially drained and converted for the most part into fertile land being currently managed by the PKB, the largest Serbian agricultural company. Crude wastewaters were being discharged into the swap by the company, resulting in the pollution of the ecosystem and, hence, the extinction of many living organisms. Tench presence was recorded in a number of bogs, the major ones including Veliko Blato (2 km²) and Reva Bog (0.4 km²). The latter is greatly affected by industrial waste waters from Krnjača settlement. The ichthyological research of the ecosystem conducted in July 1991 revealed the presence of 7 fish species, including the tench, despite the concentration of dissolved oxygen

being as low as 0.77 mgdm^{-3} (Maslić et al., 1992). This finding is a confirmation of tench tolerance to unfavourable oxygen conditions (Alabaster and Lloyd, 1980).

Central Serbia is characterised by few tench habitats registered (22–33). The hydrographic network of this part of the country is more diverse, the survival conditions being variable and generally more unfavourable. Zasavica Bog (22) is the most important tench habitat in Serbia distinguished for its preserved ambient conditions. Located in the north-western part of Serbia (Mačva region) in the flood plain of the Sava River, it is among major wildlife refuges in this part of the Balkans. The entire region (its surface area being 19.13 km^2) includes a number of bogs interconnected with canals and marsh systems with fragments of flooded meadows and forests. Zasavica Bog is protected as a Special Nature Reserve and has been included in the Ramsar List due to the high biodiversity of the entire region. The broader public became familiar with this portion of preserved nature in 2004 when the European beaver (*Castor fiber*) was reintroduced into the ecosystem after more than 100 years of its disappearance. The fish assembly composed of 23 species is particularly renowned for the presence of the mudminnow (*Umbra krameri*), a fish species classified, globally, as vulnerable (VU) (IUCN 2006) and, at national level, as critically endangered (CR) (Simić et al., 2007). The individual tench proportion in the total fish fund at certain sites ranges from 3.93–25.0%, the tench biomass percentage being 6.91–17.30% (Maletin et al., 2001). Despite the strict protection regime employed, the protected ecosystems have been endangered in the last years by the expansion of agricultural activities in the surrounding region.

The majority of other sites where the tench has been registered are characterised by negligible tench population (generally $< 1\%$ of the individual number and biomass of local ichthyofaunas). This tendency has been also recorded in popular tourist destinations in Serbia — the Sava Lake (23) on the Sava River (the area of Belgrade) and Silver Lake or Srebrno Jezero (24) along the Danube banks (near Veliko Gradište).

A low presence of tench has also been found in major Serbian hydro-electric reservoirs, the Đerdap — Dam I (25) and Đerdap Dam II (26) on the Danube River. The construction of Đerdap I dam in 1970 at the 943rd km of the river course and of Đerdap II dam in 1984 at the 863rd km, the elevation of the Danube water level by 20 m and accompanying consequences had an enormous effect on the qualitative and quantitative structure of the ichthyofauna comprising 53 species of 13 families (Dimović and Hegediš, 2002). The constructed dam is a barrier to the anadromous migrations of five acipenserid (fam. Acipenseridae) and two herring (fam. Clupeidae) species. Conversely, there has been an increase in the numbers of bream (*Abramis brama*), Asian herbivore — *C. idella*, silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Arystichthys nobilis*) (Lenhardt et al., 2004). The registered decrease in the abundance of commercially important species, such as the *C. carpio* and the tench, has been primarily induced by the depth increase leading to a drastic reduction in the surface area of the littoral zone. Moreover, the

massive increase of *C. idella* population could contribute to the reduction in the spawning areas for phytophile fish (Janković, 1998).

For recreational fishing purposes, the tench has been introduced into a number of reservoirs, including Perućica on the Drina (27), Međuvršje on the Zapadna Morava (28), Čelije on the Rasina (29), Batlava on the Batlava (30), Brestovac (31) on the Pusta Reka River, among others. The total tench population in the stated ecosystems is negligible. In terms of a potential massive increase in tench population, of particular interest are the eutrophic reservoirs Međuvršje and Čelije (located in the Zapadna Morava River Basin).

The Međuvršje reservoir is situated in the Ovčar Kablar Gorge area being protected as an Area of Superb Natural Quality (The Official Gazette of the Republic of Serbia, 5/2000). The reservoir is one of the oldest Serbian reservoirs formed in 1953 by the construction of a 31 m high dam. This resulted in the formation of a 9.3 km long lake, its maximum width being 272 m and maximum depth 12 m, the total volume amounting to $15.4 \times 10^6 \text{ m}^3$. As much as 70% of the initial volume has been deposited with river sediments over time (Lenhardt et al., 2009). The shallowing of the reservoir and the nutrient inflow have favoured the development of plankton communities and the abundance of macrophytic vegetation. The fish fauna is composed of 20 species of 7 families, the bleak (*Alburnus alburnus*) being the predominating one (Marković et al., 2007). The tench was first introduced into this ecosystem through fish stocking in 1956. Although the stockings continued (the last one being conducted in 1999 by the introduction of 100 kg of fry), the species population did not increase abundantly — the catches made in the period 2002—2006 showed that its percentage was as low as 0.27% and 0.41% of the individual and biomass level, respectively. It was induced not only by excessive catch, practiced even in the period of spawning, but also by permanent sediment deposition at spawning spots. Additionally, high variations in the water level caused by the Međuvršje hydroelectric power works led to the destruction of the roes of tench and other phytophylous species. The latest Fishery Improvement Programme for the period 2007—2011 envisages, apart from the regulation of HE power station activities, full prohibition of recreational fishing of tench over a 5-year period and the stocking of waters with fish fry (a total of 729 kg). The above measures, coupled with strict catch control measures, should contribute to a massive increase in the species population.

The Čelije Reservoir, its volume being $41 \times 10^6 \text{ m}^3$, was formed in 1979. It is specifically used for the supply of the town of Kruševac and surrounding populated areas with potable water. The lake banks have numerous bays suitable for the development of macrophyte vegetation. The fish assembly includes 17 species of 4 families, the bleak, and the goldfish (*Carassius gibelio*) predominating (Simović and Marković, 1997). Occasional tench presence has been registered (there was no record of its presence in the catch of recreational fishermen in some years). This is likely due to the massive increase in the population of pike-perch (*Sander lucioperca*), being introduced in the last decade. The reservoir has been stocked with different fish species, including the tench, on several occasions. However, the common stand in recent years has been that the tench, as a bentophagous species, should not be intro-

duced into the reservoirs used for the water supply of inhabitants. The living activities of the species being performed at the biotope bottom can lead to the mobilisation of heavy metals and other pollutants from the sediments into the water. Considering the tench abundance in this type of reservoirs in Serbia, this is quite unlikely to happen. As opposed to this, some researchers suggest that the tench introduction into eutrophic ecosystems can contribute to water quality improvement, given the fact that the mineralisation process is accelerated and that the transition of some nutrients from sediments is prevented by the tench trophic activities (Michaels, 1988). This fact, apart from the promotion of angling diversity, is the reason for the tench introduction into some carp ponds and reservoirs in Turkey (Innal and Erk'akan, 2006).

There are just a few autochthonous habitats in eastern and southern parts of Serbia. The tench is abundant in the Nišava River (southern Serbia), upstream of the city of Niš, particularly in the part of the river course passing through the Sićevac Gorge. Although the Nišava ecosystem is not a typical habitat of the species (a relatively rapid flow and stony-gravelly bottom), shallower and slower flowing parts of the river are inhabited by tench. Lake Krupac (32), a small natural lake characterised by high tench abundance, is located in this region. Being 1.5 km long, max. 200 m wide, having a silty bottom and abounding in aquatic plants, the lake is characterised by numerous populations of tench (> 10% of the individual ichthyofauna number), rudd (*Scardinius erythrophthalmus*), roach (*Rutilus rutilus*), and, frequently, pike (*Esox lucius*). The Lake catchment, used for the water supply of the town of Pirot, coupled with the low amount of precipitations in recent years, have induced both a drastic decrease in the water level and shallowing.

The Vlasina reservoir (33) is a tench habitat located at the highest altitude in Serbia (1213 m a.s.l.) (Fig. 2). By the construction of the Vlasina dam in 1949, the peat bog was converted into a 9 km long reservoir, of 3.5 km maximum width and 22 m depth, the total volume being $168 \times 10^6 \text{ m}^3$. The specific character of this ecosystem has been created by floating islands (the largest one having the area of 8 ha) being moved around with the wind. The aquatoria of the reservoir and the surrounding ground (the surface area of 32.09 km²) were included in the Ramsar List in 2007. The ichthyofauna of the reservoir includes 16 species of 5 families. Excluding the autochthonous species, the brown trout (*Salmo trutta m. fario*) and the Balkan barbel (*Barbus peloponnesius*), other species have been translocated from other waters of the Balkans. Apart from the perch (*Perca fluviatilis*) and *C. gibelio*, the tench is an abundant member of the ichthyofauna assemblage. A single sampling showed that the tench accounted for 22% of individual and 12% of the biomass percentage in the total ichthyofauna (Maletin et al., 1998b). The tench is a stable member of the fish community into which it had been introduced through stockings conducted in 1968, 1971 and 1991 (Stanković, 2000). Notwithstanding the protection measures and the organised fish guarding network employed, the ecosystems of the protected area in the last years have been endangered by the expansion of tourism and accompanying adverse activities (urbanisation, traffic, emission of different types of wastes).



Fig. 2 — Vlasina Reservoir — tench habitat at the highest altitude in Serbia

Tench in Serbian aquaculture

Up until the 1960s, the tench was the second most widespread of the cyprinid fish species in Serbia, right after the carp. The highest and long-lasting production was practised in fish ponds Ečka, Novi Kneževac and Srpski Miletić. For example, in the period 1977—1981, tench proportion in the total amount of sold fish in the then Yugoslavia ranged from 0.25—0.60% (Bojčić et al., 1982). The cyprinid fish species market in Serbia exhibits seasonal variations — the highest consumption being recorded during Saint Nicholas festive and fasting days (favouring fish of 2.5 kg or more).

The intensification of production led to changes in the fish pond preparation technique, which is followed by soil cultivation. Furthermore, the increased fish population density induced a decline in tench production. The drop in tench production resulted from the introduction of competitive herbivorous fish species, their feeding habits disrupting the tench environment. The common tench population of 5—10%, as compared to carp, was replaced by the substantial growth rate of Asian herbivores in polycultures. The indigested food excreted by carp as feces was previously used by tench and then by new consumers, the newly introduced Asian fish complex. Macrophyte vegetation, necessary for tench spawning, became food for herbivorous species.

CONCLUSION

The tench (*T. tinca*) is an insufficiently studied species of the Serbian ichthyofauna, irrespective of the global concerns and implemented introductions throughout the world. The hydroenvironmental conditions in a great number of aquatic biotopes enable the survival of the species. However, the tench population is generally a small one. The basic reasons include changes in ambient conditions and decreases in wetland surface area. The river course damming and flood zone reduction have contributed to the disappearance of the species from several natural habitats. Translocations, primarily into reservoirs, have not generally resulted in successful acclimatisation and massive abundance of the species, due to a number of abiotic and biotic factors including the competitiveness with the representatives of autochthonous ichthyofauna. The introduction of Asian herbivores to reduce the expansion of the macrophyte vegetation might have induced deterioration of spawning and feeding conditions for the phytophilous species concerned.

This study was not aimed at registering all tench-inhabited sites in Serbia, the reason being a great number of microhabitats of this cyprinid. They include, in Vojvodina, parts of the DTD Hydrosystem canal network, numerous microreservoirs, as well as dead waters (or „mrtvaja” as called in Serbia) in the catchment area of the Tisa, Begej, Tamiš and Karaš rivers. Furthermore, the study has not covered reservoirs in Central Serbia where the tench has been introduced for recreational fishing purposes as there have been no valid data on its successful acclimatisation. Additionally, the study has not registered several other sport angling fishponds formed in the last years where, apart from the carp and Asian herbivores, the tench has also been introduced. It has been also impossible to register numerous pools formed after gravel exploitation in the valleys of the Drina, Sava, Južna and Velika Morava rivers, where tench has been reported.

The conservation status of the species both in Serbia and globally is classified in the category of LR/lc (Lower Risk/least concern) (IUCN 2006). Despite the relatively wide distribution of the species in Serbia, the risk degree estimate related to the species necessitates a more detailed assessment of all relevant indicators of survival conditions (habitat alteration, population growth, exploitation rate, pollution etc.). The conservation status can be transferred into a higher category unless thorough protection measures be taken on national level.

Considering the above, the Ministry of Science of the Republic of Serbia has approved a project entitled „Reintroduction and Repopulation of Tench (*Tinca tinca*) into Fishpond Systems and Open Waters” to be implemented by experts from several university institutions in Serbia. The project envisages the introduction of high-quality spawners from a fishpond of a country with highly developed tench production. The individual fish shall be bred in the Mošorin fishpond near Novi Sad for artificial spawning purposes. The fish fry obtained shall be introduced into other fishponds and open waters. The above measures, along with more rigid protection of current habitats, should enable the recovery and increase in the tench population in Serbia.

ACKNOWLEDGMENT

The authors express their gratitude to Mirjana Lenhardt, Ph. D., Institute for Biological Research, Belgrade and Saša Branković, M.Sc., Institute for the Protection of Nature of Serbia, Niš Department, for useful information.

This study has been supported by the Ministry of Science, Republic of Serbia, Grant No. 20107.

REFERENCES

- Alabaster, J. S., Lloyd, R. (1980): *Water Quality Criteria for Freshwater Fish*. Butterworths, FAO, England.
- Anonymous (2003): *Srbija u brojkama*. Statistički zavod Republike Srbije, 5. Available via <http://www.statserb.sr.gov.yu/Pod/srb2003s.pdf>.
- Bojčić, C., Debeljak, Lj., Vuković, T., Jovanović-Kršljanin, B., Apostolski, K., Ržaničanin, B., Turk, M., Volk, S., Drecun, Đ., Habeković, D., Hristić, Đ., Fijan, N., Pažur, K., Bunjevac, I., Marošević, Đ. (1982): *Slatkovodno ribarstvo*. Poslovna zajednica slatkovodnog ribarstva Jugoslavije, Ribozajednica, Jugoslavenska medicinska naklada, Zagreb.
- Bogdanović, D. (2006): *The role of phosphorus in eutrophication*. Proc. Nat. Sci, Matica Srpska Novi Sad, 110, 75—85.
- Budakov, Lj., Maletin, S., Mučenski, V. (1983): *Prilog proučavanju ihtiofaune Obedske bare*. Drugi simpozijum o fauni SR Srbije, 119—122.
- Cakić, P., Lenhardt, M., Kolarević, J., Mićković, B., Hegediš, A. (2004): *Distribution of the Asiatic cyprinid Pseudorasbora parva in Serbia and Montenegro*. J. Fish Biol., 65(5), 1431—1434.
- Ćirković, M., Jovanović, B., Maletin, S. (2002): *Ribarstvo*. Poljoprivredni fakultet, Novi Sad.
- Dimović, D., Hegediš, A. (2002): *National Park „Đerdap”*. REC, Belgrade.
- Đukić, N., Pujin, V., Maletin, S., Gajin, S., Gantar, M., Petrović, O., Ratajac, R., Seleši, Đ. (1991): *Eutrofizacija stajaćih voda Vojvodine — I deo — postojeće stanje, trendovi i mogućnosti zaštite*. Vode Vojvodine, Novi Sad.
- Gonzales, G., Maze, R. A., Dominguez, J., Pena, J. C. (2000): *Trophic ecology of the tench in two different habitats in North-West of Spain*. Cybium, 24 (2), 123—128.
- Hegediš, A., Nikčević, M., Mićković, B. (1992): *The Fish fauna of the lower course of river Pek*. Arch. Biol. Sci., Belgrade, 44 (3—4), 11P—12P.
- Holčík, J. (1991): *Fish Introductions in Europe with Particular Reference to its Central and Eastern Part*. Can. J. Fish Aquat. Sci., 48 (1), 13—23.
- Hristić, Đ., Bunjevac, I. (1991): *Gajenje slatkovodnih riba*. Građevinska knjiga, Beograd.
- Innal, D., Erk'akan, F. (2006): *Effects of exotic and translocated fish species in the inland waters of Turkey*. Rev. Fish Biol. Fisheries, 16, 39—50.
- IUCN (2006): *IUCN Red list of Threatened Species*. Available via <http://www.iucnredlist>.

- Janković, D., Pujin, V., Hegediš, A. et al. (1994): *Community structure of the fish fauna in Danube and its tributaries*. In: Janković D., Jovičić M. (ed). The Danube in Yugoslavia — contamination, protection and exploitation, Institute for Biological Research „Siniša Stanković”, Belgrade, 131—148.
- Janković, D. (1998): *Natural reproduction by Asiatic herbivorous fishes in the Yugoslav section of the River Danube*. Ital. J. Zool., 65 (2), 227—228.
- Karadžić, B., Mijović, A. (2007): *Environment in Serbia: an indicator — based review*. Serbia Environmental Protection Agency, CICERO, Belgrade.
- Kostić, D., Maletin, S. (1992): *Contribution to the knowledge of ichthyofauna of some stagnant waters in Vojvodina*. Ichthyologia, 24 (1), 25—31.
- Lenhardt, M., Cakić, P., Kolarević, J. (2004): *Influence of the HEPS Đerdap I and Đerdap II dam construction on catch of economically important fish species in the Danube River*. Ecohydrol. Hydrobiol., 4 (4), 499—502.
- Lenhardt, M., Hegediš, A., Cvijanović, G., Jarić, I., Gačić, Z., Mićković, B. (2006): *Non-native freshwater fishes in Serbia and their impacts to native fish species and ecosystems*. Geoph. Res. Abst., European Geosciences Union, Vol. 8, 07727.
- Lenhardt, M., Marković, G., Gačić, Z. (2009): *Decline in the Index of Biotic Integrity of the Fish Assemblage as a Response to Reservoir Aging*. Water Resour. Manage., 23, 1713—1723.
- Maletin, S., Đukić, N., Obradović, S., Ivanc, A., Miljanović, B., Pujin, V., Zhenjun, S. (1996): *Heavy metal content of fish communities inhabiting the Yugoslav section of the River Danube*. Arch. Hydrobiol. Suppl. 113, Large Rivers, 10 (1—4), 535—540.
- Maletin, S., Đukić, N., Miljanović, B., Ivanc, B. (1997): *Status of allochthonous ichthyofauna of Panonian basin in Yugoslavia*. Ekologija, 32 (2), 87—98.
- Maletin, S., Đukić, N., Miljanović, B., Teodorović, I. (1998a): *Kontrola procesa eutrofizacije primenom biomanipulacije*. Konferencija „Zaštita voda '98”, Kotor 291—296.
- Maletin, S., Đukić, N., Miljanović, B., Teodorović, I. (1998b): *Contribution to knowledge of the ichthyofauna of the Vlasina Reservoir*. Ichthyologija, 30 (1), 83—85.
- Maletin, S., Miljanović, B., Đukić, N., Teodorović, I. (2001): *Naselje riba u specijalnom rezervatu prirode Zasavica*. U Igić, R., Gajin, S. (ed). Zasavica 2001. PMF, Institut za Biologiju, Goransko-Ekološko Društvo, Sremska Mitrovica, 70—75.
- Maletin, S., Čirković, M., Jurakić, Z. (2005): *Conservation and improvement of diversity and production of fish fund in canals of Hydrosistem Danube-Tisa-Danube*. Savremena poljoprivreda, 54 (1—2), 119—124.
- Maletin, S., Neatnica, G. (2006): *Elaborat o kvalitet voda i stanju ribljeg fonda u kanalima Hidrosistema DTD*. Poljoprivredni fakultet i JVP Vode Vojvodine, Novi Sad.
- Maletin, S., Stojanović, S., Mišković, M. (2007a): *Monitoring program — Suzbijanje prekomernog razvoja vodene vegetacije Osnovne kanalske mreže hidrosistema Dunav — Tisa — Dunav u Bačkoj belim amurom*. Poljoprivredni fakultet, Department za stočarstvo, Novi Sad.

- Maletin, S., Stojanović, S., Mišković, M. (2007b): *Kontrola razvoja vodene makrofitske vegetacije belim amurom*. III Međunarodna Konferencija „Ribarstvo”, Beograd, 274—280.
- Marković, G., Simović, S. (1994): *Ichthyofauna of the middle course of Zapadna Morava river as a saprobic indicator*. Arch. Biol. Sci., Belgrade, 46 (1—2), 39—44.
- Marković, G., Lenhardt, M. (2007): *Teški metali u ribama Zapadne Morave*. III Međunarodna Konferencija „Ribarstvo”, Beograd, 287—290.
- Marković, S. G., Simić, M. V., Ostojić, M. A., Simić, B. S. (2007): *Seasonal variation in nutrition of chub (Leuciscus cephalus L., Cyprinidae, Osteichthyes) in one reservoir of West Serbia*. Proc. Nat. Sci, Matica Srpska Novi Sad, 112, 107—113.
- Marković, Z., Poleksić, V., Mitrović-Tutundžić, V. (2007): *Akvakultura u Srbiji*. III Međunarodna Konferencija „Ribarstvo”, Beograd, 35—40.
- Maslić, M., Obuškov, Lj., Cakić, P., Jakovčev, D., Lenhardt, M., Kalafatić, V., Topalović, V. (1992): *Efekti permanentnog zagađenja na jedan barski ekosistem*. Konferencija „Zaštita voda '93”, 274—278.
- Michaels, V. K. (1988): *Carp farming*. Henry Ling Ltd The Dorset Press, Dorchester.
- Ostojić, A., Čurčić, S., Čomić, Lj., Topuzović, M. (2005): *Estimation of the Eutrophication Process in the Gruža Reservoir (Serbia and Montenegro)*. Acta hydrochim. Hydrobiol., 33 (6), 1—9.
- Pančić, J. (1860): *Pisces Serbiae*. Glasnik Društva Srpske Slovesnosti, Beograd 12, 500—667.
- Ramsar Sites Database (2008): *Ramsar List of Wetlands of International Importance*. Available via http://www.ramsar.org/key_cp.e.htm.
- Simić, V., Simić, S., Paunović, M., Cakić, P. (2007): *Model of the assessment of the critical risk of extinction and the priorities of protection of endangered aquatic species at the national level*. Biodivers. Conserv., 16, 2471—2493.
- Simonović, P., Nikolić, V. (1997): *Freshwater fish of Serbia: an annotated check list with some faunistic and zoogeographic considerations*. Bios, 4, 137—156.
- Simonović, P. (2006): *Ribe Srbije*. NNK International, Biološki fakultet, Beograd.
- Simonović, P., Marić, S., Nikolić, V. (2006): *Occurrence of paddlefish Polyodon spathula (Walbaum, 1792) in the Serbian part of the lower River Danube*. Aquat. Invas., 1 (3), 183—185.
- Simović, S., Marković, G. (1997): *Novi podaci o ihtiofauni akumulacija Centralne Srbije*. III Simpozijum „Ribarstvo Jugoslavije”, Cetinje—Rijeka Crnojevića, 73—80.
- Stanković, S. (2000): *Jezera Srbije — limnološka monografija*. Srpsko geografsko društvo, Beograd.
- Šipoš, S., Pankov, N., Mijić, I., Jurca, T., Miljanović, B. (2007): *Ihtiofauna vodotoka Jegrička*. III Međunarodna Konferencija „Ribarstvo”, Beograd, 291—297.
- Vuković, T., Ivanović, B. (1971): *Slatkovodne ribe Jugoslavije*. Zemaljski muzej BiH, Sarajevo.
- Wheeler, A. (1978): *Key to the Fishes of Northern Europe*. Warner Ltd, London.

Wright, R. M., Giles, N. (1991): *The population biology of tench, Tinca tinca (L.) in two gravel pit lakes*. J. Fish Biol., 38 (1), 17—28.

ПРИЛОГ ПОДАЦИМА О РАСПРОСТРАЊЕЊУ ЛИЊАКА
(*TINCA TINCA* L., CYPRINIDAE, PISCES) У СРБИЈИ

Горан С. Марковић¹, Мирослав А. Ђирковић²,
Стеван А. Малетин², Николина Ј. Милошевић²

¹ Агрономски факултет Чачак, Универзитет у Крагујевцу,
Цара Душана 34, 32000 Чачак, Србија

² Пољопривредни факултет Нови Сад, Универзитет у Новом Саду,
Трг Д. Обрадовића 8, 21000 Нови Сад, Србија

Резиме

Испитивања ихтиофауне Србије која су до сада извршена указују на спорадично присуство лињака (*Tinca tinca* L.) у типичним стаништима. Хидроеколошки услови непходни за опстанак ове рибље врсте веома су променљиви и, генерално, повољнији у северним крајевима земље (Војводина). Иако је присуство лињака регистровано на више од 30 локација, постоји тенденција смањивања популационе бројности. Разлог за то су смањење површине плавних зона обрадних макрофитском вегетацијом настало услед преграђивања и регулисања речних токова, велика варирања водостаја и смањење количине воде у већини водоотокова, омасовљење алохтоних фитофагних врста, таложење наноса у акумулацијама, погоршање квалитета воде и други чиниоци. Степен угрожености лињака у Србији званично је означен као статус Ниског ризика — Најмања угроженост. Овај статус може прећи у вишу категорију уколико се настави тенденција погоршања услова опстанка врсте.

Marko J. Tucakov

Institute of Nature Conservation of Serbia, Radnička 20a, 21000 Novi Sad, Serbia

SPECIES COMPOSITION AND NUMBER OF BREEDING BIRDS IN URBAN PARKS IN NOVI SAD

ABSTRACT: Numbers of breeding pairs of breeding birds and breeding density are established by territory mapping in Limanski (12.9 ha), Futoški (8.26 ha), and Dunavski (3.9 ha) Parks in Novi Sad from early March to late June 2007. In Limanski Park 11 species of breeders were found, the most numerous being tree sparrow (*Passer montanus*). Futoški Park had 15 breeders and the most numerous were great tit (*Parus major*). The smallest number of species (9) bred in Dunavski Park, with great tit as the most common breeder. The greatest density of breeding pairs was recorded in the largest park (37.7 pairs/ km²). Hole-breeders dominated in Futoški and Dunavski Park, but their number was the same as the number of canopy-breeders in Limanski Park. Measured with Sorensen index of similarity, the greatest similarity was found between breeding avifauna of Futoški and Dunavski Park (72), then between Futoški and Limanski (61), while the smallest similarity was found if we compare breeding avifauna of Dunavski and Limanski Park. Compared with breeding avifauna in other parks in Central Europe, breeding avifauna of Novi Sad parks is relatively poor.

KEYWORDS: breeding birds, Novi Sad, parks

INTRODUCTION

Natural plant communities in cities are typically highly modified by urbanization. Much vegetation is eradicated and replaced by construction areas leaving less growing space for plants, while the composition and structure of remaining vegetation is also altered. One of the most prominent plant communities in cities are „planted communities” characterized by active human efforts to plant trees, scrubs and other vegetation. Some native species during the forming of these communities can be retained, but they mainly consist of new plantings, many of which are non-native species. Urban development destroys habitat for many species, modifies habitat for others and creates new habitat for some species (Adams et al., 2005).

Despite the efforts to study birds in European cities (Kelcey and Rheinwald, 2005), breeding birds in urban parks in cities of Serbia are very rarely studied up to now. The only exceptions are published list of bree-

ders of Belgrade parks and other green areas (Vasić & Stevanović, 1972) and list of breeding birds of Futoški Park (Nemeth, 1989) and Palić Park (Hullo, 1998—1999), but without any quantitative data.

The aim of this paper was to present the composition of breeding bird fauna of parks in Novi Sad, their numbers, and to compare these parameters with the breeding birds in parks in other European cities.

MATERIAL AND METHODS

Research was done in Novi Sad, the second largest city of Serbia. In the most recent official census from 2002, the city had an urban population of 216.583 <http://en.wikipedia.org>. The city is located on the border of the Bačka and Srem regions, on the banks of the Danube river and Danube-Tisa-Danube Canal, and it faces the northern slopes of Fruška Gora mountain. It is founded in 1694. The urban area is 129.4 km² and the urban population density is 1673.7 inhabitants/km². It is bounded by extensive agricultural areas to the north and east, the Danube on the west and south.

Three urban parks were taken in consideration: Limanski, Futoški, and Dunavski (Table 1). These are the only urban parks in Novi Sad maintained and used by people on the regular basis. Human activities include grass mowing, flower planting, tree and bush cutting and maintenance, recreation, walking with dogs, maintenance of paths, benches, and children playing grounds. All parks are situated within the urban matrix, surrounded by high buildings, houses, and streets. The total surface of tree parks makes 1.93% of the urban area of Novi Sad.

Tab. 1 — Characteristics of urban parks in Novi Sad

Park	Origin	Dominant trees	Dominant bushes	Data source
Limanski (12.9 ha)	1950— 1960	<i>Populus x euramericana</i> , <i>P. alba</i> , <i>P. canescens</i> , <i>Salix alba</i> , <i>S. babylonica</i> , <i>Pinus nigra</i>	<i>Forsythia suspensa</i>	www.zelenilo.co.yu, own data
Futoški (8.26 ha)	1900— 1910	<i>Corylus colurna</i> , <i>P. nigra</i> , <i>P. silvestris</i> , <i>Sophora japonica</i> , <i>Quercus robur</i> , <i>Populus alba</i> , <i>Taxodium distichum</i>	<i>Juniperus horizontalis</i> , <i>J. Chinensis</i>	Popović and Korać, 2005
Dunavski (3.9 ha)	1895	<i>P. nigra</i> , <i>Thuja orientalis</i> , <i>Betula alba</i> , <i>C. colurna</i> , <i>Celtis occidentalis</i> , <i>Abies concolor</i>	<i>Ligustrum ovalifolium</i> , <i>J. horizontalis</i> , <i>J. prostrata</i> , <i>J. sabina</i> , <i>Spirea x vanhouttei</i>	Đaković, 1998

The highest diversity of trees and bushes was found in Futoški Park, followed by Dunavski Park. These parks are remnants of once vast forest areas occurred on the place where urban area is situated currently. Limanski Park was created much later. Dunavski and Futoški parks are designated as nature monuments (Anonymous, 1998, Anonymous 2005).

Numbers of breeding pairs of breeding birds were established following the method of territory mapping (Sutherland, 1996). Parks were visited

eight and seven times respectively (from early March to late June) during the breeding season of 2007. Single visits were done in the early morning, from 6 to 9 a.m.

Level of similarities of breeding avifauna was controlled using Sorensen index of similarity: $QS = 2c/(a + b) \times 100$ where C is the common part of two sets, A is the size of the first set and B is the size of the second set (Tomiało *et al.*, 1984).

RESULTS

In Limanski Park 11 species bred. Tree sparrow (*Passer montanus*) was the most numerous. Fifteen breeders were recorded in Futoški Park, the most numerous being great tit *Parus major*. The smallest number of species (9) bred in Dunavski Park, with great tit as the most common breeder. The greatest density of breeding pairs was recorded in the largest park (Table 2).

Tab. 2 — Number of pairs and density of breeding birds of urban parks in Novi Sad

Species	Limanski	p/10 ha	Futoški	p/10 ha	Dunavski	p/10 ha
<i>Passer montanus</i>	10	7.7	3	3.6	2	5.1
<i>Parus major</i>	6	4.6	4	4.8	3	7.7
<i>Sylvia atricapilla</i>	1	0.8	3	3.6	1	2.6
<i>Columba palumbus</i>	2	1.6	3	3.6	2	5.1
<i>Pica pica</i>	1	0.8	1	1.2	2	5.1
<i>Corvus corone cornix</i>	4	3.1	1	1.2	0	0
<i>Sturnus vulgaris</i>	3	2.3	4	4.8	0	0
<i>Streptopelia decaocto</i>	0	0	4	4.8	1	2.6
<i>Asio otus</i>	0	0	1	1.2	1	2.6
<i>Fringilla coelebs</i>	4	3.1	0	0	0	0
<i>Picus viridis</i>	2	1.5	0	0	0	0
<i>Dendrocopos major</i>	1	0.8	0	0	0	0
<i>Muscicapa striata</i>	1	0.8	0	0	0	0
<i>Passer domesticus</i>	0	0	2	2.4	0	0
<i>Accipiter nisus</i>	0	0	1	1.2	0	0
<i>Carduelis chloris</i>	0	0	1	1.2	0	0
<i>Phenicurus ochruros</i>	0	0	1	1.2	0	0
<i>Dendrocopos major</i>	0	0	1	1.2	0	0
<i>Serinus serinus</i>	0	0	1	1.2	0	0
<i>Dendrocopos syriacus</i>	0	0	0	0	1	2.6
<i>Carduelis chloris</i>	0	0	0	0	1	2.6
Total	35	27.1	31	37.7	14	35.9

Hole-breeders dominated in Futoški and Dunavski Park, but their number was the same as the number of canopy-breeders in Limanski Park (Figure 1).

The greatest similarity of breeding avifauna was found between Futoški and Dunavski Park (72) and between Futoški and Limanski (61). The smallest similarity was found if we compare breeding avifauna of particular parks of Dunavski and Limanski Parks, using Sorensen index of similarity.

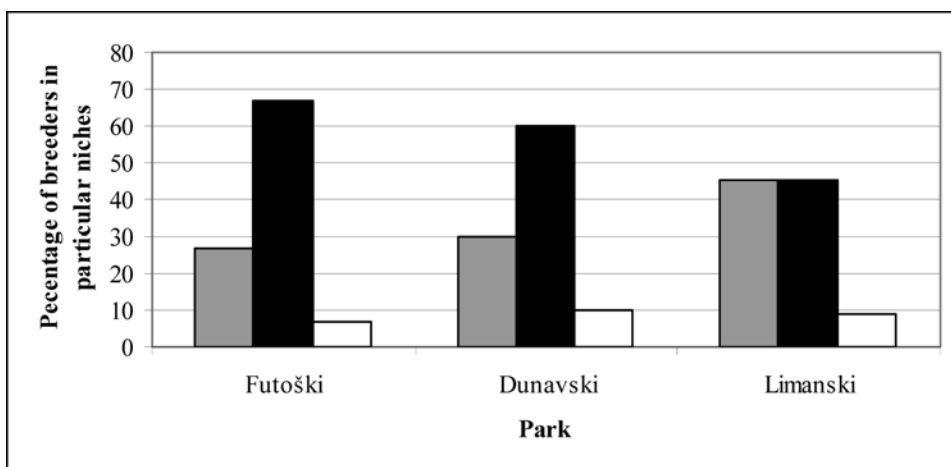


Fig. 1 — Share of ground-breeders (white), canopy-breeders (gray), and hole-breeders (black) in total number of breeding birds of urban parks in Novi Sad

DISCUSSION

In comparison with the bird fauna of Prague parks (Fuchs et al., 2002), parks in Novi Sad were inhabited by relatively poor number of species. Overall breeding density (Table 2) was much lower than recorded in Prague, where 21–206 pairs/10 ha bred, respectively (Fuchs et al., 2002). There are several reasons for this. Studied parks in Novi Sad are surrounded by streets and buildings and are isolated from natural forests. On the other hand, all parks are relatively small and intensively managed, which, for example in Limanski Park, excludes bushes and ground cover, but includes relatively high abundance of dogs (own data).

There are no recent studies of birds of urban parks in Serbia. Species account done in Futoški Park in 1981–1988 resulted in only 10 breeders: *P. pica*, *P. major*, *T. merula*, *S. atricapilla*, *S. vulgaris*, *P. domesticus*, *P. montanus*, *C. coccythraustes*, *C. chloris*, and *C. carduelis* (Nemeth, 1989). Out of these, two species did not breed in the park in 2007, but eight species colonized it since then: *C. palumbus*, *C. c. cornix*, *S. decaocto*, *A. otus*, *A. nisus*, *P. ochruros*, and *S. serinus* (Table 2). Long-eared owl *A. otus* had adapted its breeding habits and became regular urban breeder in Novi Sad in the meantime (Radišić and Stojšin, 2001). That was the case also with black redstart *P. ochruros*, the species that inhabited Novi Sad starting from 1987, when first breeding males were registered in Petrovaradin (Purger, 1988/89). Sparrow hawk *A. nisus* is a new breeding raptor for Novi Sad (Puzović and Grubač, 2000). Favorable conditions for its breeding exist due to planted black pines *Pinus nigra* in Futoški Park. Such sites sparrow hawk inhabits also in urban parks of Belgrade (Puzović & Grubač, 2000). All other newcomers are expected and became rather widespread in urban areas in recent decades (pers. data). However, despite it bred in Futoški Park in the period

1981—1988 (Nemeth, 1989), blackbird *T. merula* was not found in Novi Sad parks in 2007. It breeds in neighboring Fruška Gora frequently (Stojnić, 2009). It was not found breeding on other sites in Novi Sad, outside of parks (pers. data). Reason for this remained unknown, having in mind that blackbird is common urban breeder in all larger cities in Serbia, for example: Belgrade (Vasić and Stevanović, 1972), Subotica, Sombor (own data). In 6 out of 12 studied parks in Prague blackbird was the most numerous species with the greatest density (Fuchs et al., 2002; Table 3), which was also the case in cemeteries of Bratislava (Kocian et al., 2003).

Comparison with data obtained in smaller Belgrade parks in 1960's, when 25 breeding species were recorded (Vasić and Stevanović, 1972) shows that there are 12 species breeding in parks in both cities, while only 6 are breeding only in Novi Sad (*C. palumbus*, *A. otus*, *P. viridis*, *P. domesticus*, *A. nisus*, *P. ochruros*), while 13 are breeding only in Belgrade. However, historical changes in breeding avifauna in cities are very fast (Vasić, 1970), and current breeding birds community might be completely different.

Tab. 3 — Comparison of breeding avifauna in Novi Sad with Czech parks

Park	Size (ha)	Number of breeders	The most numerous species	Density of the most numerous species (p/ha)
Prague (Fuchs et al 2002)				
Krčský les	?	23	<i>P. major</i>	5.8
Grebovka	?	20	<i>F. coelebs</i>	20.2
Riegrový sady	?	16	<i>C. chloris</i>	18.1
Kralovská obora S	?	34	<i>T. merula</i>	10.2
Petrín	?	23	<i>T. merula</i>	26.0
Nový židovský hřbitov	?	16	<i>T. merula</i>	21.3
Karlovo and karlinske namešti	?	8	<i>S. decaocto</i>	30.6
Olšanské hřbitovy	?	17	<i>F. coelebs</i>	23.9
Lesík na Jarově	?	17	<i>T. philomelos</i>	48.0
Vitkov	?	12	<i>T. merula</i>	25.4
Rumište v Malešicích	?	7	<i>T. merula</i>	12.5
Cisarský ostrov	?	32	<i>T. merula</i>	25.2
Česke Pardubice (Vranová et al., 2007)				
Bubeníkovy sady	3.2	22	<i>S. decaocto</i>	21.9
Park Na Špici	2.5	20	<i>Phylloscopus collybita</i>	36.0
Park Pod Vinicí	3.4	23	<i>F. coelebs</i>	23.7
Tyršovy sady	10.5	22	<i>S. atricapilla</i> and <i>F. coelebs</i>	8.6 respectively
Novi Sad (this paper)				
Limanski	12.9	11	<i>P. montanus</i>	7.7
Futoški	8.26	15	<i>P. major</i> , <i>S. vulgaris</i> and <i>S. decaocto</i>	4.8 respectively
Dunavski	3.9	9	<i>P. major</i>	7.7

Ground-nesting species are poor colonizers of urban parks (Jokimäki, 1999), which were proven for Novi Sad parks, as well. Ground in parks is co-

vered by short grass, managed very intensively, and occupied by humans and domestic animals (potential predators), which disable successful breeding on the ground and low bushes.

The greatest similarity between breeding communities of Futoški and Dunavski Parks is most probably a result of similar breeding conditions. Limanski Park, in comparison with other two, offers poorer breeding condition, despite it is the largest: there are almost no bushes in it and the trees (poplars) are of similar age and equally dispersed. Breeding conditions in recent time could be even worsened as a result of current massive cutting of trees (in winter 2007/2008) and building of concrete roads thru the park. Therefore, potential changes in breeding avifauna in this and other parks should be carefully monitored.

REFERENCES

- Adams, L. W., Van Druff, L. W., Luniak, M. (2005): *Managing urban habitats and wildlife*. In: Braun, C. E. (ed.) *Techniques for wildlife investigations and management*. 6th edition. Wyd.: The Wildlife Society, Bethesda, Maryland, USA, pp. 714—739.
- Anonymous (2005): *Odluka o zaštiti Futoškog parka*. Službeni list Grada Novog Sada 18: 491—494. In Serbian.
- Anonymous (1998): *Uredba o zaštiti spomenika prirode „Dunavski park”*. Službeni glasnik RS 25: 690—691. In Serbian.
- Đaković, N. (1998): *„Dunavski park” — spomenik prirode*. Zavod za zaštitu prirode Srbije, Beograd. In Serbian.
- Fuchs, R., Škopek, J., Formánek, J., Exnerová, A. (2002): *Atlas hnízdního rozšíření ptáků Prahy*. Česká společnost ornitologická, Praha.
- Gibbons, W. D., Hill, D., Sutherland, J. W. (1996): *Birds*. Pp. 227—259. In: Sutherland, J. W. (ed.): *Ecological Census Techniques*. Cambridge University Press, Cambridge.
- Hullo, I. (1998—1999): *Prirodne vrednosti Paličkog parka sa ornitološkog aspekta*. Ludaški zapisi 2—3: 85—95. In Serbian.
- Jokimäki, J. (1999): *Occurrence of breeding bird species in urban parks: Effects of park structure and broad-scale variables*. *Urban Ecosystems* 3(1): 21—34.
- Kelcey, J. G., & Rheinwald, G. (2005): *Birds in European Cities*. Ginster Verlag, St. Katharinen.
- Kocian, L., Némethová, D., Melicherová, D., Matušková, A. (2003): *Breeding bird communities in three cemeteries in the City of Bratislava (Slovakia)*. *Folia Zoologica* 52 (2): 177—188.
- Nemeth, G. (1989): *Data on birds of Futoški Park in Novi Sad*. *Ciconia* 1: 22—25.
- Popović, M., Korać, J. (2005): *Spomenik prirode „Futoški park u Novom Sadu” — predlog za stavljanje pod zaštitu kao značajno prirodno dobro*. Zavod za zaštitu prirode Srbije, Beograd. In Serbian.
- Purger, J. J. (1988/89): *Present range of black redstart, *Phoenicurus ochruros gibraltariensis* (Gmelin), 1789 in Vojvodina and surrounding areas*. *Bulletin of Natural History Museum, Belgrade* B43/44: 175—183. In Serbian with English summary.

- Puzović, S., Grubač, B. (2000): *Sparrowhawk Accipiter nissus*. pp. 93—98. In: Puzović, S. (ed.): *Atlas of Breeding Birds of Prey of Serbia*. Institute for Protection of Nature of Serbia, Belgrade.
- Radišić, D., Stojšin, A. (2001): *Sova utina (Asio otus): životni uslovi u urbanoj sredini Novog Sada*. Ciconia 10: 128—135. In Serbian with English summary.
- Stojnić, N. (2008): *Distribution of breeding birds in forest habitats of Fruška Gora*. MSc thesis. University of Novi Sad, Faculty of Natural Sciences and Mathematics, Department of Biology and Ecology, Novi Sad.
- Tomiałojć, L., Wesolowski, T., Walankiewicz, W. (1984): *Breeding bird community of a primeval temperate forest (Białowieża National Park, Poland)*. Acta ornithologica 20: 243—310.
- Vasić, V. (1970): *Ptice Beograda — faunistički spisak*. Larus 21—22: 130—136. In: Serbian with German summary.
- Vasić, V., Stevanović, V. (1972): *Karakteristike ornitofaune užeg područja Beograda*. Larus 24: 115—127. In Serbian with German summary.
- Vranova, S., Lemberk, V., Hampl, R. (2007): *Ptáci Pardubic*. Východočeská pobočka České společnosti ornitologické & Východočeské muzeum v Pardubicích, České Pardubice. In Czech.

САСТАВ ВРСТА И БРОЈНОСТ ПТИЦА ГНЕЗДАРИЦА У ГРАДСКИМ ПАРКОВИМА У НОВОМ САДУ

Марко Туцаков

Завод за заштиту природе Србије, Радничка 20а, 21000 Нови Сад;
e-mail: mtucakov@eunet.rs

Резиме

Састав врста и бројност птица гнездачица установљени су методом мапирања територија у Лиманском (12,9 ha), Футошком (8,26 ha) и Дунавском (3,9 ha) парку у Новом Саду у периоду од раног марта до касног јуна 2007. године. У Лиманском парку гнездило се 11 врста, а најзаступљенија међу њима била је полски врабац *Passer montanus*. У Футошком парку гнездило се 15 врста птица. Врста са највећом густином била је велика сеница *Parus major*. Најмањи број врста (9) забележен је на гнежђењу у Дунавском парку, у којем је такође велика сеница била најзаступљенија гнездачица. Највећа укупна густина птица гнездачица забележена је у највећем парку, Лиманском (37,7 pairs/km²). Птице дупљашице доминирале су међу гнездачицама Футошког и Дунавског парка, а њихов број је био једнак као и број врста које се гнезде у крошњама у Лиманском парку. Мерена Соренсеновим индексом сличности, највећа сличност установљена је између заједнице птица гнездачица Футошког и Дунавског парка (72), затим следи сличност авифауна Футошког и Лиманског (61), док је најмања сличност била између авифауна Дунавског и Лиманског парка. У поређењу са фауном птица гнездачица других паркова у средњој Европи, може се закључити да је фауна гнездачица паркова у Новом Саду релативно сиромашна.

*Dragan R. Žikić¹, Gordana M. Ušćebrka¹,
Dušan S. Gledić², Miodrag I. Lazarević²*

¹ Faculty of Agriculture, University of Novi Sad, Serbia

² Faculty of Veterinary Medicine, University of Belgrade, Serbia

THE INFLUENCE OF LONG TERM SOUND STRESS ON HISTOLOGICAL STRUCTURE OF IMMUNE ORGANS IN BROILER CHICKENS

ABSTRACT: The aim of this paper was to examine the effect of different duration sound stress on immune organs of broiler chickens of different age. Nine groups, with 10 chickens in each group were included in experiment. The histological structure of bursa of Fabricius, thymus, and spleen were analyzed. The results indicated that the bursa of Fabricius, in relation to the other examined organs, was the most sensitive to this kind of stress. Histological changes of spleen and thymus were also observed, but less prominent except in chickens after more than 30 days of exposure to stress. According to our results, degree of histological changes of immune organs under the influence of sound stress depends on the length of exposure and age of chickens.

KEY WORDS: sound stress, broiler chickens, immune organs

INTRODUCTION

Stress is the reaction of the organism to stimulus, which disturbs physiological equilibrium, usually with harmful consequences. This disturbance of homeostasis has resulted in changes in the concentration of large number of different hormones that have a crucial role in the regulation of immune system function.

The link between neuroendocrine and the immune system, is first reflected in the existence of receptors for a number of chemical mediators in immune cells. These chemical modulators, actually hormones, are ACTH, glucocorticosteroids, vasoactive intestinal peptide (VIP), substance P, prolactin, growth hormone, sex steroids, catecholamine, acetylcholine, releasing hormones, and opioid molecules (K h a n s a r i et al., 1990).

Immunomodulatory effect of these hormones can be direct, when they affect biochemical reactions that are responsible for cell proliferation, differentiation, and their functions. In addition, indirect influence is reflected in the

modulation of production and/or activities of lymphokines and monokines (Munck et al., 1984; Johnson and Torres, 1985; Ooi et al., 1987).

Furthermore, in stress reaction autonomic nerve system (ANS) is activated. Importance of that system is in his connection with lymphoid organs, which are innervated by ANS. In that way, ANS is involved in process of immunomodulation (Ackerman et al., 1989; Ackerman et al., 1991; Felten and Felten, 1991; Vizi et al., 1995).

Results of different investigations show that stress in broiler chickens has significant influence on immune system and immune organs. Lazarević et al. (2000) reported that sound stress has immunosuppressive effects on broiler chickens. Heat stress has also led to significant immunosuppression in broiler chickens (Al-Ghamdi, 2008). Social stress induces decreasing ratio between weight of bursa of Fabricius and body weight (Mohamed and Hanson, 1980). Investigating the nutritional stress on the bursa of Fabricius and thymus of chickens Griffiths et al. (1985) point out that stress induces thymus atrophy and reduction in bursa of Fabricius weight. Chickens exposed to stress promoted by vaccination and overpopulation had smaller weight of the bursa of Fabricius, thymus and spleen compared to control group (Awadalla, 1998). Pardue et al. (1985) investigated influence of high temperature on broiler chickens and point out that thermal stress decreased weight of bursa of Fabricius and spleen. ACTH infusion induces decreasing of bursa of Fabricius weight in chicken. Same response of that organ was observed in *Gallus domesticus* after implantation of pellets with corticosterone and cholesterol (Davison et al., 1985). In addition, exogenous cortisol and treatment with combination of cortisol and thermal stress decrease Bursa of Fabricius and spleen weight in broiler chickens (Brake et al., 1988).

The aim of our investigation was to estimate the influence of long-term sound stress on morphology of immune organs (thymus, spleen, and Bursa of Fabricius) in broiler chickens.

MATERIALS AND METHODS

The experiments were conducted on 90 HYBRO broiler chickens, divided into nine groups (each group consisting of 10 birds) as follows:

- C_{1–15} — control (non exposed) group
- O_{1–15} — chickens exposed to the sound stress from 1st to 15th day of life
- C_{1–30} — control (non exposed) group
- O_{1–30} — chickens exposed to the sound stress from 1st to 30th day of life
- O_{15–30} — chickens exposed to the sound stress from 15th to 30th day of life
- C_{1–45} — control (non exposed) group
- O_{1–45} — chickens exposed to the sound stress from 1st to 45th day of life
- O_{15–45} — chickens exposed to the sound stress from 15th to 45th day of life
- O_{30–45} — chickens exposed to the sound stress from 30th to 45th day of life

The control and exposed chickens were kept in two different buildings under same conditions of temperature, light, humidity, and number of birds per m². They were fed *ad libitum* and had free access to water. According to the experimental schedule, chickens were moved from the building without noise to the building where they were subjected to sound stress. The chickens were exposed to sound stress in sound attenuated building using a fire alarm bell (95 dB) for stress sessions lasted 120 min., every day. The bell was programmed to ring for 5 sec in a variable interval schedule (5 to 115 s) through signals generated by PC software.

Every 15 day, birds were sacrificed as show in above schedule. For histological investigation samples of thymus, spleen and bursa of Fabricius were fixed in Bouin solution for 24 h, and after standard histological procedure of dehydration, organs are embedded in paraffin. Serial cuts, 5 µm thickness, were made by microtome and stained with hematoxylin and eosin. Histological analysis was performed with light microscope Leica DLMS connected with camera (Leica DC-300). Software for image analysis was IM 1000 (Leica Imaging Systems Ltd, Cambridge, UK).

RESULTS

Bursa of Fabricius

The typical changes of bursa of Fabricius in all groups of chickens exposed to sound stress are reflected in the follicles atrophy and increase of connective tissue (Figure 1A-F).

The degree of changes was in direct correlation with the duration of exposure to sound stress. In the group that was exposed to stress from day 1 to day 45 a complete atrophy of lymph follicles, the large amount of connective tissue and appearance of cysts in epithelium was observed.

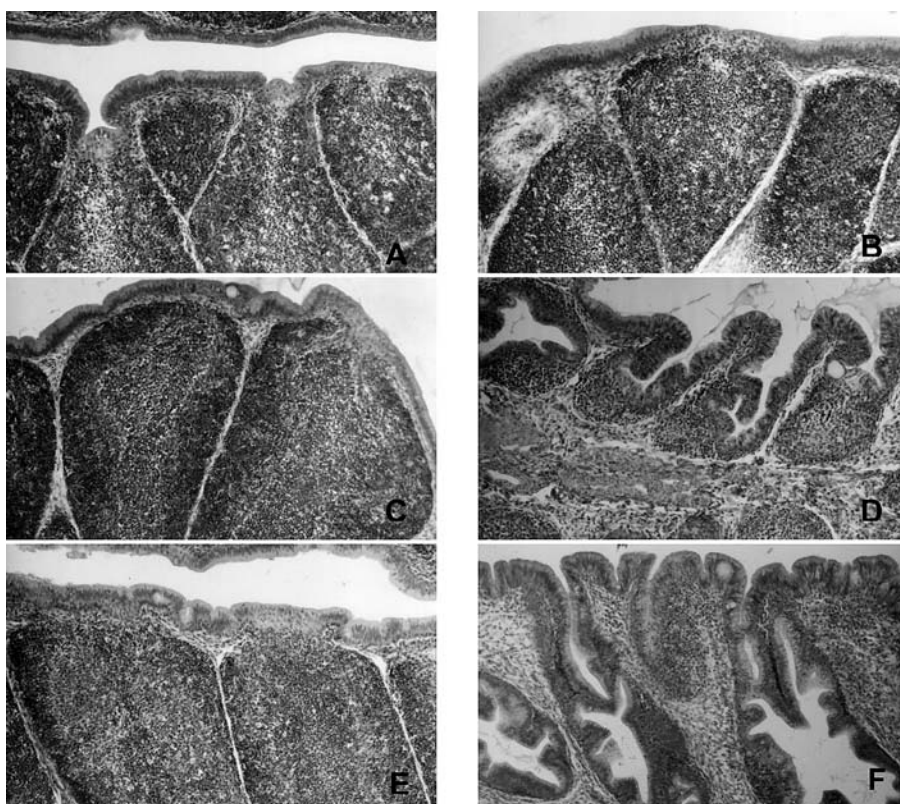


Fig. 1 — Microphotographs of chicken bursa of Fabricius (A — control group, 15 days old; B — sound stress from day 1 to day 15; C — control group, 30 days old; D — sound stress from day 1 to day 30; E — control group, 45 days old; F — sound stress from day 1 to day 45 (HE; 150x)

Thymus

The chronic action of sound stress did not cause significant changes in the histological structure of thymus. Discrete changes are observed only in the group of chickens that was exposed to chronic sound stress from 1st to 30th day of life (Figure 2A-B). Thymus of these animals is characterized by the presence of a larger number of macrophages in the cortex and the more developed connective tissue, but the basic structure of organ was unchanged.

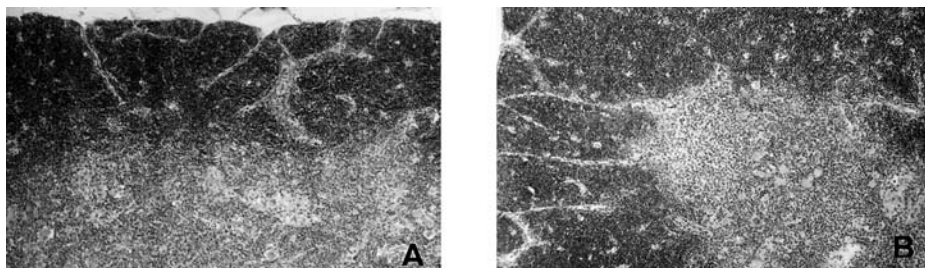


Fig. 2 — Thymus of 30-day-old chicken. Control (A) and chicken exposed to sound stress from day 1 to day 30 (B) (HE; 150x)

Spleen

The changes in histological structure of chicken spleen exposure to chronic sound stress, like in thymus, were not significantly expressed. At the largest number of chickens, spleen was without changes and the follicles development degree was in accordance with their age (Figure 3). The only change was observed in chickens exposed to sound stress from day 1 to day 30. Spleen of these animals was characterized by a smaller number of follicles and less developed of periarteriolar lymphoid sheath (PALS) (Figure 3).

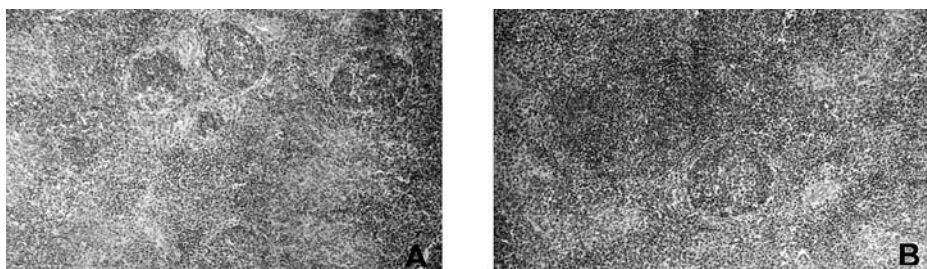


Fig. 3 — Spleen of 30 day old chicken (A) and spleen of chicken exposed to sound stress from 1st to 30th day of age (B) (HE; 150x)

DISCUSSION

Histological analysis of broiler chickens immune organs showed significant differences in structure of bursa of Fabricius in chicken groups exposed to chronic sound stress. In younger chickens, changes were slightly expressed and reflected in the better-developed connective tissue. However, in older chickens, changes were intensive, and reflected in the progressive reduction of the lymphoid follicles size, increasing of connective tissue amount and appearance of the cyst in both epithelium and in the follicle.

On the base of our findings could be concluded that bursa of Fabricius is very sensitive organ to the sound stress. In accordance with our findings are

the results of other authors who have examined the impact of social stress (Mohamed and Hanson, 1980), nutritive stress (Griffiths et al., 1985), stress caused by vaccination (Awadalla, 1998) and thermal stress (Pardue et al., 1985; Brake et al., 1988) on the bursa of Fabricius; they point out that stress induces atrophy of that organ which was reflected by organ weight decreasing. Stock densities, also, have effects on the bursa of Fabricius structure (Muniz et al., 2006).

Our results point out that duration of stress has significant influence on degree of histological changes. What was obvious in comparison of its histological changes in groups exposed to stress 15, 30, and 45 days. Higher degree of follicular atrophy was in groups that were exposed to sound stress in longer period. In addition, age of chicken had influence at the degree of histological changes of bursa of Fabricius. At the same duration of sound stress, older compared to younger chickens have much more prominent structural changes of the bursa of Fabricius.

These results confirm that the sound stress causes immunosuppression. We assume that the basic mechanism of action of all kind of stressors on the immune system is basically the same, what result in immunosuppressive action of glucocorticoids on this system.

Researching the effects of glucocorticoids on the bursa of Fabricius, Compton et al. (1990) found that dexamethasone causes regression and reduction in its cell activity. These authors also found that the regression of bursa of Fabricius was a consequence, first of programmed cell death (apoptosis) activated by steroids, and second as a consequence of the migration of lymphocytes from the tissue caused by action of dexamethasone.

Unlike the bursa of Fabricius, chronic sound stress caused less prominent changes of thymus structure. Higher degree of follicular atrophy was noticed in chickens longer exposed to sound stress. In thymus tissue were found the larger number of macrophages and higher amount of connective tissue in chickens of the age of 30 days than in control animals.

Atrophy of chicken thymus, which is in connection with reduction of organ weight, is found in nutritive stress (Griffiths et al., 1985). Reduction of thymus weight was also found in the chickens exposed to stress due to vaccination and overpopulation (Awadalla, 1998). These finding indicate that stress affect the thymus, as the primary immune organ, but that the degree of its change depends of animal strain, type of stressor, the length of exposure, etc.

Increase of macrophages number and increase in amount of connective tissue in chickens thymus in our experiment indicate that the thymus react to stress. These findings are in accordance with the results of Compton et al. (1990), which indicate the important role of macrophages in the inactivation of lymphocytes after glucocorticoides treatment.

Like in thymus, changes in the spleen structure of chicken exposed to sound stress were not significantly expressed. In the largest number of animal, spleen normal structure is preserved, and development of follicles is in accordance with age of chickens.

The only deviation in spleen structure in chickens exposed to sound stress observed at the age of 30 days. In these animals, spleen is characterized by a

smaller number of follicles and less developed PALS. Stress caused by vaccination and overpopulation, as well as high temperature, were resulted in the reduction of the spleen weight of broiler chickens (Pardue et al, 1985; Awadalla, 1998). Researching the impact of stress on the spleen, Davison et al. (1985) found that ACTH infusion causes reduction of spleen weight. In addition, treatment with cortisol and cortisol in combination with the heat stress causes the reduction of body mass in broiler chickens (Brake et al., 1988).

Histological changes in the spleen showed a lower sensitivity of this organ on sound stress compared to the Bursa of Fabricius, which is in fully agree with the results of experiments that are performed by Compton et al. (1990). They showed that organs where mature lymphocytes were located are less sensitive on the influence of glucocorticoides.

CONCLUSION

Chronic sound stress causing significant changes in histological structure of bursa of Fabricius in broiler chickens. These changes are reflected in the follicles atrophy, development of connective tissue and appearance of cysts in epithelium and in the follicles.

In broiler, thymus exposed to sound stress, minor changes are visible, exactly increasing number of macrophage and more connective tissue then in control group at 30 days of age.

In the same period, minor changes were observed in the spleen structure of broiler chickens exposed to sound stress then in control group. These changes are shown by smaller number of follicles and less developed of periarteriolar lymphoid sheath (PALS).

REFERENCES

- Ackerman, K. D., Felten, S. Y., Dijkstra, C. D., Livnat, S., Felten, D. L. (1989): *Parallel development of noradrenergic innervation and cellular compartmentation in the rat spleen*. Exp Neurol, 103: 239—255.
- Ackerman, K. D., Bellinger, D. L., Felten, S. Y., Felten, D. L. (1991): *Ontogeny and senescence of noradrenergic innervation of rodent and spleen*. In: Ader, R., Felten, D. L., Cohen, N., editors. Psychoneuroimmunology (2nd ed), New York, Academic press, 71—126.
- Al-Ghamdi, Z. H. (2008): *Effects of Commutative Heat Stress on Immunoresponses in Broiler Chickens Reared in Closed System*. Int J Poult Sci, 7: 964—968.
- Awadalla, S. F. (1998): *Effect of some stressors on pathogenicity of Eimeria tenella in broiler chicken*. J Egypt Soc Parasitol, 28: 683—690.
- Brake, N. P., Brake, J., Thaxto, J. P., Murray, D. L. (1988): *Effect of cortisol on cutaneous basophil hypersensitivity to phytohemagglutinin in chickens*. Poultry Sci, 67: 669—673.

- Compton, M. M., Gibbs, P. S., Johnson, L. R. (1990): *Glucocorticoid activation of deoxyribonucleic acid degradation in Bursal lymphocytes*. Poultry Sci, 69: 1292—1298.
- Davison, T. F., Freeman, B. M., Rea, J. (1985): *Effects of continuous treatment with synthetic ACTH1-24 or corticosterone on immature Gallus domesticus*. Gen Comp Endocrinol, 59: 416—423.
- Felten, S. Y., Felten, D. L. (1991): *Innervation of lymphoid tissue*. In: Ader, R., Felten, D. L., Cohen, N., editors. Psychoneuroimmunology (2nd ed), New York, Academic press, 27—70.
- Griffiths, G. L., Singh, U. M., Hopkins, D., Wilcox, G. E. (1985): *Nutritional stress as a cause of thymic atrophy in broiler chickens*. Avian Dis, 29: 103—106.
- Johnson, H. M., Torres, B. A. (1985): *Regulation of lymphokine production by arginine vasopressin and oxytocin: modulation of lymphocyte function by neurohypophyseal hormones*. J Immunol, 135 (2 Suppl): 773s—775s.
- Khansari, D. A., Murgó, A. J., Faith, R. E. (1990): *Effects of stress on the immune system*. Immunology Today, 11: 170—175.
- Lazarević, M., Žikić, D., Ušćebrka, G. (2000): *The influence of long term sound stress on the blood leukocyte count, heterophil/lymphocyte ratio and cutaneous basophil hypersensitive reaction to phytohemagglutinin in broiler chickens*. Acta Vet — Beograd, 50: 63—76.
- Mohamed, M. A., Hanson, R. P. (1980) *Effect of social stress on Newcastle disease virus (LaSota) infection*. Avian Dis, 24: 908—915.
- Munck, A., Guyre, P. M., Holbrook, N. J. (1984): *Physiological functions of glucocorticoids in stress and their relation to pharmacological actions*. Endocr Rev, 5: 25—44.
- Muniz, E. C., Fascina, V. B., Pires, P. P., Carrijo, A. S., Guimarães, E. B. (2006): *Histomorphology of Bursa of Fabricius: Effects of Stock Densities on Commercial Broilers*. Brazilian J Poult Sci, 8: 217—220.
- Ooi, B. S., MacCarthy, E. P., Hsu, A. (1987): *Beta-endorphin amplifies the effect of interleukin-1 on mouse mesangial cell proliferation*. J Lab Clin Med, 110: 159—163.
- Pardue, S. L., Thaxton, J. P., Brake, J. (1985): *Role of ascorbic acid in chicks exposed to high environmental temperature*. J Appl Physiol, 58: 1511—1516.
- Vizi, E. S., Orsó, E., Osipenko, O. N., Haskó, G., Elenkov, I. J. (1995): *Neurochemical, electrophysiological and immunocytochemical evidence for a noradrenergic link between the sympathetic nervous system and thymocytes*. Neuroscience, 68: 1263—1276.

УТИЦАЈ ДУГОТРАЈНОГ ЗВУЧНОГ СТРЕСА НА ХИСТОЛОШКУ ГРАЂУ ИМУНОЛОШКИХ ОРГАНА КОД БРОЛЕРСКИХ ПИЛИЋА

Драган Р. Жикић¹, Гордана М. Ушћебрка¹,
Душан С. Гледић², Миодраг И. Лазаревић²

¹ Пољопривредни факултет, Универзитет у Новом Саду, Србија

² Факултет ветеринарске медицине, Универзитет у Београду, Србија

Резиме

Циљ рада је да се испита утицај звучног стресора на имунолошке органе бројлера који су били излагани у различитим узрастима и различитом трајању стресора. Формирано је девет група, а свака група је имала по 10 пилића. Испитивана је хистолошка грађа Фабрицијеве бурзе, грудне жлезде и слезине. Резултати указују да је Фабрицијева бурза, у односу на остале испитиване органе, најосетљивија на деловање ове врсте стресора. Промене се уочавају и код грудне жлезде и слезине, али су мање и јављају се након излагања стресору више од 30 дана. Степен промена у хистолошкој грађи имунолошких органа под утицајем звучног стресора зависи од дужине излагања као и узраста пилића.